2015

Identifying population structure and local adaptation in the American lobster using behavioral, morphometric, and genetic techniques

BOSTON UNIVERSITY

GRADUATE SCHOOL OF ARTS AND SCIENCES

Dissertation

IDENTIFYING POPULATION STRUCTURE AND LOCAL ADAPTATION IN THE AMERICAN LOBSTER USING BEHAVIORAL, MORPHOMETRIC, AND GENETIC TECHNIQUES

by

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B.S., Old Dominion University 2009

Submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

2015

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DEDICATION

To my wife and editor-in-chief, Makayla, who continues to inspire me each day and has supported me throughout this process. Without her love, encouragement, and much needed prodding during the difficult times, I would not have been able to produce this work or maintain my sanity. I am eternally grateful. Much love, muy mucho.

ACKNOWLEDGMENTS

Thank you to my advisor, Jelle Atema, without whom I would not be where I am today, and who supported me in science but also in my educational endeavors. Many thanks to my dissertation committee; Sean Mullen, Christopher Schneider, Les Kaufman, Suchi Gopal, Fred Wasserman. Thank you to the many members of the Atema lab, especially John Majoris, Kristin Radcliffe, Ashley Jennings, David Minkoff, Adrienne Lohe, and the many undergraduate researchers integral to the work accomplished. Thanks, also, to our collaborators Laura Benestan, Louis Bernatchez, Gabi Gerlach, and Janna Deppermann.

Thank you to Angela Seliga who mentored me and gave me opportunities to progress in my teaching and curriculum development and worked as hard as anybody to push me to succeed. Thank you to Rebecca Lewis, Bennett Goldberg, Fred Wasserman, Kathryn Spilios, Allison Cox, Reggie Jean, Mike Dennehy and the many others who contributed to my path in science education.

Finally, I want to thank my wonderful family who supported me unequivocally throughout my education: my beautiful wife, Makayla, my parents, Dave and Lisa, my in-laws, Scott and Danita, my brother, Christopher, and my grandparents, aunt, and uncles who worked in unison to inspire and encourage me during my education and career. Also to Liam, our rescued border collie, who graciously sacrificed his playtime to allow me to write.

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ABSTRACT

The seeming lack of barriers to gene flow in the northwest Atlantic ocean has led to the general assumption that the population of the American lobster (*Homarus americanus*) is largely panmictic. However, morphological and genetic data presented in this dissertation suggest that lobster populations are less homogenous than once believed with potential for behavioral barriers to mating and selection of locally adaptive traits. Additionally, both long-term fishing pressures and the recent spread of a destructive epizootic shell disease may have impacted population structure. We developed a novel photographic technique to rapidly collect accurate morphological data with the ability to maintain a database of images for the purposes of re-sampling and testing additional hypotheses. During this study, we found significant morphometric differences between samples of lobsters from collection sites as close as 25km apart. Morphological differences may have originated due to differential selection or plastic responses to environmental variation. To analyze population genetic structure, I surveyed genetic variation using RADseq. Analysis of 1614 putatively neutral SNPs found little genetic difference (Average F_{ST}=0.00137) between sample sites suggesting a high level of gene

flow between regions. Several additional markers appeared to be under divergent selection between sample sites. A genome scan analysis of both neutral SNPs and SNPs under selection found several selected SNPs associated with principal components of morphological characters. A subsequent BLAST analysis identified a number of the selected SNPs lying in the *H. americanus* transcriptome, suggesting functional importance. Further experimentation is required to quantify the impacts of plasticity or local adaption in the origin of morphological differences between lobster populations, although the significant differences identified in this research are likely due to a combination of the two. The overarching conclusion is that lobster populations are, in fact, more differentiated than previously predicted and, as such, the findings presented here may have significant management implications.

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DISSERTATION INTRODUCTION

The American lobster (*Homarus americanus*) is a commercially important decapod crustacean that supports a major fishing industry in the United States and Canada with 2013 global landings totaling 140 million tons (Fig. 1) and with 2013 US landings of nearly 68 tons worth a value of over $450 million (NOAA Commercial Fisheries Statistics). Recently, catch rates have increased dramatically as lobster populations in the Gulf of Maine have grown. As such a large fishery, active management that is updated to fit the most up-to-date understanding of the species is of great importance so as to ensure its long-term health (Fogarty 1995; Henry & Johnson 2015). With respect to informing management, recent research has utilized novel and higher resolution next-generation approaches to determine genetic and demographic connectivity of marine populations (Palsboll et al. 2007; Palumbi et al. 2003; Allendorf et al. 2010). This information is especially important in commercially exploited fisheries

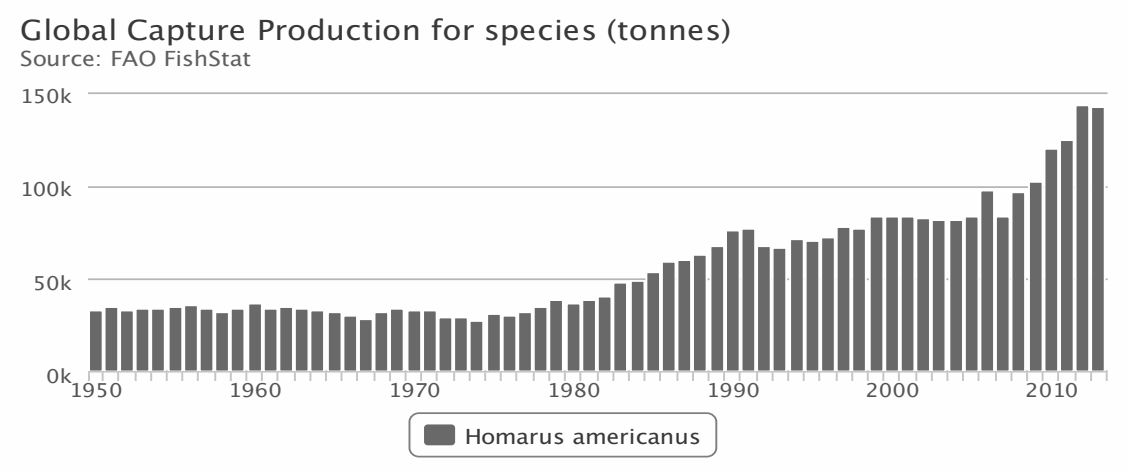


Figure 1. Global lobster (*H. americanus*) catch in tons ranging from 1950 to 2013. Source: FAO FishStat and NOAA Fisheries.

where fishing pressure can have dramatic impacts on stock health and, in the event of a population decline, the resulting recovery (reviewed in Ciannelli et al. 2013).

The second chapter of this dissertation deals with olfactory recognition and behavioral avoidance of epizootic shell disease. The research described examines whether female lobsters recognize and then avoid males that are infected with epizootic shell disease, a rapidly spreading disease in which chitinivorous bacteria degrade an individual's carapace, rendering it weakened and at higher risk of death (Cobb & Castro 2006). The disease can change the biochemical profile of lobsters and could potentially change their urine and other body odors (Laufer et al. 2005; Castro et al. 2005; Tarrant et al. 2012). In turn, this may affect social responses, including avoidance of diseased animals. Behavioral avoidance could reduce the spread of disease (Behringer et al. 2006). We conducted odor choice tests with pairs of (size- and site-matched) healthy and shell-diseased males. The results showed that healthy intermolt females did not discriminate between the odor of healthy or diseased males. In addition, we investigated the effect of shell disease on male dominance in size-matched males. Healthy males established dominance over shell-diseased males in 15 of 18 fights. Subsequent choice tests with the same male pairs again showed no significant difference between the time females spent with healthy versus diseased males, but they preferred dominant males slightly. Because most dominant males were healthy, it confers a slight advantage to healthy males. The results were similar for animals from 2 subpopulations, each with considerable incidence of shell disease. Behavioral disease avoidance mechanisms were not seen and may have not yet evolved, if this disease is a recent phenomenon. Also, the disease may be caused

more by environmental conditions than by genetic predisposition or interanimal contact, making disease recognition irrelevant.

The third, fourth, and fifth chapters address questions regarding genetic and morphometric population structure in *Homarus americanus*. In 2007, the Atema and Gerlach laboratories began a joint project to determine whether lobsters showed genetic and morphometric population structure and whether there was any evidence of olfactory recognition and preference by females for males from their own region over males from other regions. The preliminary study showed both genetic and morphometric differences between samples from Maine and Rhode Island and behavioral odor choice flume experiments showed at females recognized, through olfactory cues, males from their own region and showed preference for associating with those males in the flume. These results led to the question as to how wide-spread were these genetic, morphometric, and behavioral differences and led to a wide-spread sampling of individuals from sites in Rhode Island, New Hampshire, Maine, and from canyons located offshore on the edge of the continental shelf. Some sites showed genetic differences at microsatellite loci and every sampled site showed significant morphometric structure through a comprehensive examination of morphology using 63 different measurements of each individual. However, the high degree of morphometric structure was not commensurate with the low level of genetic structure leading to the major questions addressed within the final three chapters of this dissertation.

1) Is there a more efficient way to measure lobsters reliably and in a way that can allow for increased sample sizes, re-sampling, and testing different hypotheses?
2) Do lobsters exhibit neutral genetic population structure that was not accounted for in the microsatellite analysis but that could explain the significant morphometric structure?
3) Are there loci that, through a RADseq study and association tests with morphometric characters, can be identified as candidates for genomic regions of local adaptation?

These questions are each addressed separately in chapters 3, 4, and 5 of this dissertation.

Chapter 3 discusses how, in an effort to better discriminate morphologically among lobsters from different sites, we developed a photographic method using ImageJ and compared it with commonly used "hand" measurements. We standardized the measuring process using a strap-down board for both dorsal and ventral photographs with a camera mounted at a fixed position above the lobster. Discriminant analysis showed that both hand and photographic methods were useful in discriminating lobsters — both males and females — from three different sites. Additionally, the photographic method improved reproducibility and resolution, it reduced measurement time at the dock, and it created a permanent record for later verification, additional statistical analyses, and observer training.

Chapter 4 details a RADseq approach to identifying genetic population structure that showed no statistically significant genetic structure; however, pairwise genetic

differences between sites were similar to other studies that did find statistically significant differences (Benestan et al. 2015; Kenchington et al. 2009) , possibly a result of our smaller sample sizes. The low levels of genetic differentiation between sites in this study failed to explain the morphometric differences between sites. As such, the next step was to identify whether we could identify any candidate loci for local adaptation that could explain some greater level of the morphometric differences.

In Chapter 5, the identification and exploration of a number of candidate loci for local adaptation are examined through outlier and association analyses. Results of these analyses identified a number of SNP markers that were putatively under selection and a number that were significantly associated with morphometric differences. Of these markers, only one was found to be both putatively under selection and significantly associated with morphology. In humans, this marker is associated with cell growth and division (Naora et al. 1998; Volarevic et al. 2000) and, although we have no evidence of a corresponding role in *H. americanus*, it, along with other candidate loci identified through this study, provide a reasonable next-step for further experimentation.

The sum of this research suggests that lobsters exist in structured populations and, although the genetic differences are relatively small, adaptive differences in regions of the genome in addition to plastic responses could account for the identified morphometric differences. Population structure has direct implications for management of commercially exploited fisheries due to the ability and need to predict the impacts of fishery collapse due to overfishing or disease (Palumbi et al. 2003; Brown et al. 1995). It also informs the

development of marine protected areas and preserves (Gell 2003; Ryman et al. 1995). It is, therefore, of critical importance to continue the investigations described in this text.

NO OLFACTORY RECOGNITION OF SHELL DISEASE IN *H. Americanus*

Introduction

Epizootic shell disease (ESD) is a serious issue facing inshore populations of American lobster, *Homarus americanus* (Milne Edwards), in southern New England (SNE) waters. The lesions indicative of general shell disease infection (ISD) were first discovered on lobsters kept in high-density tidal impoundments (Hess 1937). In the wild lobster populations, there is a low level endemic form of shell disease that occurs possibly as a result of injuries. In 1996, a new form, called epizootic shell disease (ESD) was described for lobsters from Rhode Island and southern Massachusetts (Cobb & Castro 2006; Castro & Angell 2000; Glenn & Pugh 2006). ESD can be differentiated from other forms of shell disease through the pathology of the lesions. Smolowitz et al. (2005) confirmed that the pathology of the lesions differed from other shell disease types in that pillars of chitin remained while degradation occurred in the other polymers in the carapace. Kunkel et al. (2012) discussed that lesions in ISD are located at dermal gland canals, while ESD lesions are located on the plane between these canals. The complex bacterial community in ESD lesions has now been well characterized through several methods (Bell et al. 2012; Chistoserdov et al. 2012; Meres et al. 2012). Two bacterial species in particular, *Aquimarina 'homaria'* and '*Thalassobius'* sp. are abundant in the lesions in wild ESD lobsters (Chistoserdov et al. 2012).

Castro et al. (2006) found a mean prevalence of over 45% in the upper East Passage of Narragansett Bay in Rhode Island with disease prevalence fluctuating seasonally with the molting season. Greatest prevalence is in the late fall after the molt

occurs and the shells may still be soft. Most vulnerable are egg-bearing females, which molt least frequently and are hormonally distinct. Research on the behavioral effects of shell disease in *H. americanus* is limited. A tagging study showed no difference between diseased and healthy lobsters in migration distance or direction (Landers 2005). Time-budgets of healthy and diseased lobsters showed that diseased lobsters spent significantly more time in contact with shelter materials indicating a behavioral difference between healthy and shell diseased individuals (Castro et al. 2005).

Lobsters with ESD were found to have higher levels of ecdysone (molting hormone) than non-shell diseased lobsters, indicating the involvement of the endocrine system in the defense strategy (Laufer et al. 2005). Tarrant et al. (2012) found that the expression of several genes changed significantly with disease status. In animals showing signs of ESD, low arginine kinase expression in muscle indicates that lobsters may be energetically compromised. There was an elevated expression of ecdysteroid receptor in both muscle and hepatopancreas of shell diseased lobsters and increased cytochrome P450 enzymes indicating that shell disease is associated with disruption of chemical metabolism and hormonal signaling (Tarrant et al. 2012). Homerding et al. (2012) found that lobsters from the eastern portion of Long Island Sound (ELIS) had compromised immune systems relative to lobsters from western Long Island Sound (WLIS) or from Maine, suggesting that differences in the immune status of lobsters could be a factor in the regional differences in disease prevalence (prevalence is significantly higher in ELIS than WLIS or Maine). Lobsters with ESD showed significant differences in plasma antimicrobial activity in plasma and hemocyte phagocytosis, oxidative burst in

hemocytes as well as bacterial load in the hemolymph. All these physiological disruptions could affect the chemical makeup of an individual's urine. Lobsters use urine in chemical communication (Atema & Steinbach 2007). This provides the possibility for olfactory recognition of a diseased animal.

Intraspecific avoidance of diseased animals could reduce the spread of the disease if it were transmitted from animal to animal through physical contact such as demonstrated by healthy Caribbean spiny lobsters (*Panulirus argus,* Latreille), which actively avoided individuals infected with *P. argus* virus 1 (PaV1), a lethal disease, even before the infected individual showed any symptoms of the disease (Behringer et al. 2006). The study concluded that chemical cues released by infected lobsters mediated the avoidance behaviors of healthy lobsters.

Lobsters use urine to send pheromones to conspecifics during fights (Breithaupt & Atema 2000). The urine signals are received by aesthetasc sensilla of the antennules (Johnson & Atema 2005) and serve in individual recognition (C. Karavanich & Atema 1998; CHRISTA Karavanich & Atema 1998), which is important in dominance relationships for both sexes. Male dominance, in turn, is related to mating success (Cowan & Atema 1990). While pre-molt females significantly preferred dominant males ($p = 0.01$), inter-molt females showed only slight preference for dominant males ($p = 0.07$) (Bushmann & Atema 2000). In sum, lobsters use odor signals to recognize sex, dominance, and individuals.

Female lobster's preference for males may be affected by shell disease. First, females could detect the presence of the disease and avoid males that emit that cue,

similar to the phenomenon found in *P. argus*. Second, in the event healthy males are better capable of establishing dominance over diseased males, females may select indirectly for healthy individuals by preferring the dominant male.

Since male dominance is important for female mate choice an inability to gain dominance could negatively affect reproductive success in diseased males. While females with their longer intermolt stage suffer greater disease incidence, a lack of healthy males -such as in diseased areas- may further depress local reproduction. Using pre-molt rather than inter-molt females might increase their mate choice and might more directly relate to reproduction and disease transmission. However, it would be nearly impossible to have sufficient numbers of healthy pre-molt females available while at the same time having the right combination of size-matched healthy and diseased males from the same site. Therefore we chose to work with size-matched, primarily intermolt males and females.

Our research was motivated by the fact that *H. americanus* relies on chemical signals to obtain information about its environment, prominently including its social environment (Atema & Steinbach 2007). The nephropore gland of the lobster contains glyco-proteins and its ducts lead into the urine bladder (Bushmann & Atema 1996). Lobster urine contains large amounts of proteins (100-300 mg/ml, (McLaughlin et al. 1999), which could serve as pheromones. The effects of shell disease on hemolymph protein levels or other metabolic disruptions (Floreto et al. 2000) could alter the chemical signals used by lobsters during mating or fighting, thus affecting behavioral responses.

Here we investigate the behavioral response of intermolt females when presented with the odor of a healthy versus a diseased male, both before and after the pair had

established a dominance relationship. The effect of shell disease on male dominance was also evaluated.

Methodology

Lobsters for behavioral tests were collected from two sample locations in Rhode Island where ESD is prevalent; from the Upper East Passage area inside Narragansett Bay (referred to as the RIN population) or an adjacent 2 x 5 mile area outside the Bay in Rhode Island Sound (the RIS population). The sites are approximately 25 miles apart. Data collected for each lobster include carapace length (CL), sex, and degree of shell disease. Male lobster CL ranged from 77.5-88.5 mm in RIN and 82.6-85.5 mm in RIS, allowing for the formation of size-matched pairs (within 2 mm CL). Female CL ranged from 81.8-93.0 mm in RIN and 81.5-86.0 mm in RIS. Degree of ESD in males ranged from 40-80% of the shell surface exhibiting lesions; however, testing the effect of different degrees of shell disease was not within the scope of this study. Healthy males and females that showed signs of ESD and animals that molted were immediately removed from the experiment and replaced if a suitable replacement was available.

All behavioral experiments were conducted in the fall of 2008, winter 2009 and spring 2010, in the Boston University Marine Program Laboratory. Similar to natural conditions, temperature of the artificial seawater ranged from 15-17°C with a mean of 16°C, salinity ranged from 32-33 ppt, and pH was held at a mean of 8.0. All lobsters were fed three times per week on a diet of peeled shrimp, which was chosen to maintain water quality in the system. The experimental design required holding animals for long periods in the lab. Initially, male lobsters were separated into individual bins while female

lobsters were kept together in larger tanks. Individual recognition experiments (C. Karavanich & Atema 1998) showed that lobsters no longer recognize each other after 2 weeks of separation. Thus, to avoid dominance and individual recognition effects in the first test series, we separated the males for at least 2 weeks in individual holding bins after we obtained them.

For odor choice experiments we used a dimly lit, two-channel flume containing two shelters upstream and one shelter downstream (Fig. 2). The flume measured 350 x 135 cm with a water depth of 28 cm upstream and 34 cm downstream. The mean flow was ~2 cm/sec, re-circulated and filtered through activated carbon to reduce lingering scents. The males served as odor "donors" and for tests were held in the upstream shelters; the female was acclimated in the downstream shelter. A solid plastic barrier separated the upstream half of the flume; the downstream half had no barrier. Water passed through the male shelters and flowed down in two separate columns toward the female shelter converging upon a central drain behind the female's shelter (Fig. 2). Daily dye tests ensured that the two water masses remained unidirectional and distinct until reaching the female's shelter. The experimental design called for two test series: first when the males of a pair were unfamiliar with each other (naïve) and then after they had established a dominance relationship.

To avoid the possibility that lobsters prefer to associate with conspecifics from their own site, females from each site were tested only with males from their own site. We tested the male pairs first before and then after they had established dominance. The experimental design allowed us to evaluate effects of disease, male dominance, and their

interaction on female recognition and preference for male odors, while avoiding effects of site preference. The behavioral experimental design called for the RIN and RIS populations to be tested separately, each with 10 size-matched male pairs (one healthy, one shell diseased) evaluated by 10 healthy females. Difficulty of simultaneously obtaining the proper number of healthy and diseased males and females from the two sites led to three separate testing periods. In each period several animals had to be replaced. A suitable replacement would be an animal of the same sex that was size matched, of the same disease condition, and from the same sample site as the individual to be replaced.

It was not always possible to obtain individuals from the proper site and in the proper condition when needed. Therefore we chose to work with size-matched, primarily intermolt males and females. As a result, 10 male pairs from RIN and 8 from RIS were used as well as 30 RIN and 24 RIS females. Of the 10 RIN pairs, 5 were tested with 10 females (50 trials, n = 50), the remaining 5 pairs with 5-8 females (33 trials, n = 33). A total of 83 trials (n = 83) were run using RIN individuals. The 8 RIS pairs all were tested with 10 females resulting in a sample size of 80 trials (n = 80). Combining the populations resulted in a sample size of 163 trials (n = 163).

Initial testing was two males and one female in their respective shelters. After a five minute acclimation period in which the odor plumes emanating from the males were established, the trial began: the female's shelter was opened and lit by an overhead (40W) light that encouraged her to leave. For ten minutes the location of the female within the flume was measured with the following distance criteria: time spent in her shelter (SH),

in the “open” flume area (O, downstream of the central barrier), in the “channel” (C, past the central barrier but not within 30 cm of the male shelters), “near” (N, within 30 cm of a male shelter, but not trying to enter the shelter), and “entering” (E, actively attempting to enter the partially blocked shelter) (Fig. 2). Thus, OCNE represents the summated time on either left or right side of the flume.

After ten minutes, the female was gently coaxed back into her shelter and the males were switched between sides of the flume to account for inadvertent side bias. After a five-minute acclimation to re-establish the odor plumes and re-acclimate the female she was again released for an identical ten-minute choice trial. For the subsequent test, the two males remained unchanged while the female was exchanged to repeat the same process until all available healthy females from the proper site were tested. We calculated trial results by combining the two parts of the test period. Using the statistical program JMP, a Wilcoxon signed rank test was used to evaluate whether there was a significant difference between the time females spent within the odor plumes of the healthy or diseased males. No difference in time spent in either male’s odor would be expected if females showed no preference for healthy or shell diseased males.

After the naïve trials were run with the entire female panel, each male pair was placed in an observation tank (size 0.9 x 0.6 x 0.4 m) where they established dominance; we used dominance criteria adapted from Atema and Voigt (1995) and Karavanich and Atema (1998). After the fight, the two males were held in the tank overnight for continuous contact and consolidation of the dominance relationship. The following day

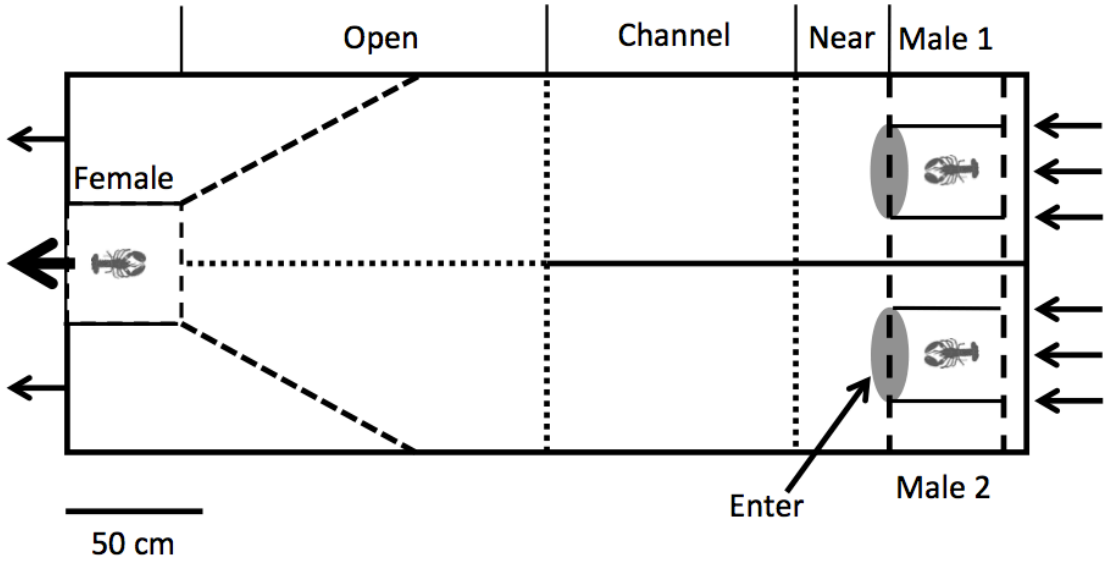


Figure 2. Diagram of odor choice flow tank. Female in downstream start box; one healthy and one shell diseased male in –barricaded- upstream shelters; lobsters incl. claws drawn to scale; Open, Channel, Near, Enter: tank areas used to evaluate female choice; "Enter" is defined as a behavior at the shelter entrance (see text). Broken lines: grating barriers allowing water flow through. Dotted lines: indicate tank areas (no physical barriers). Arrows: water flow. See text for details.

the same female lobster panel was used to express possible preference for a member of the male pair, post-fight.

Results

Regardless of distance criterion used in the analysis, there was no significant difference between the amount of time intermolt females from either population (RIN, RIS) or both populations combined (RIN+RIS) spent with pre or post-fight healthy and shell diseased males (Table 1). The Wilcoxon signed-rank values (W, H-SD) were predominantly positive suggesting a trend toward preference for healthy males. In the post-RIN analysis female enter attempts (E) showed a trend toward preference for healthy males (E: $W = 68$; $p = 0.068$). However, the latter result can be explained by a dominance effect.

Dominance was established by all 10 healthy RIN males and by 5 out of the 8 RIS males; the other 3 dominant RIS males were shell-diseased (RIN+RIS, two-tailed binomial test, $p = 0.008$). Females preferred dominant males by one criterion only: time spent trying to enter (E) dominant male shelters. Despite short durations of effort and a small sample size, this was significant for the RIS population (E: Tdom = 9, Tsub = 6; W, D-S = 114; $p = 0.031$), and nearly significant for the RIN population and the RIN and RIS populations combined (Table 2). The RIS females showed a trend toward healthy dominant males also for the OCNE criterion where the time values are more robust (OCNE: Tdom = 651, Tsub = 536; W, D-S = 364; $p = 0.059$). In the dominance analysis (Table 2), Wilcoxon values were positive except in the analysis of dominant, shell-diseased males (Post-RIS, SD-Dom; n = 30) indicating slight female preference for healthy males. These results were not significant and did not affect our conclusion: inter-molt females do not prefer the odor of healthy or shell-diseased males. However, females do recognize male dominance odor as expressed in a small increase in entering attempts of the dominant males' shelter.

Discussion

The results showed that intermolt female lobsters have no significant preference for healthy or shell diseased males; both RIN and RIS females showed the same lack of preference. This suggests lack of disease recognition. The results are in some respect Nevertheless, the result that shell disease is not recognized by odor is robust. Lack of female preference for healthy males in both RIN and RIS populations was abundantly

Table 1. Shell Disease odor recognition: Results and Statistical evaluation. Female preference for Healthy (H) vs Shell Diseased (SD) males by population site (RIN, RIS, RIN + RIS combined) and by experimental phase (PRE and POST fight), each with sample size of male pairs; n: number of female choice trials; flume areas: defined in text; H and SD: mean time (s) females spent on side of Healthy vs Shell Diseased male (in seconds ± SEM); W (H-SD): Wilcoxon signed rank; p: significance (in bold type: values near p=0.05).

H/SD	n	Flume area	H (s)	SD (s)	W (H-SD) (s)	p
PRE-RIN	83	OCNE	552±27	592±27	-95	0.6
(10 male pairs)		CNE	314±28	352±33	-81	0.6
		NE	203±23	243±29	-110	0.5
		E	12±4	8±3	22	0.5
PRE-RIS	80	OCNE	638±24	562±24	309	0.12
(8 male pairs)		CNE	399±28	340±27	280	0.18
		NE	294±27	258±26	177	0.4
		E	17±5	14 ±4	132	0.3
PRE-(RIN+RIS)	163	OCNE	594±18	577±18	423	0.5
(18 male pairs)		CNE	356±20	346±21	331	0.5
		NE	248±18	250±20	-91	0.9
		E	15±3	11±3	251	0.2
POST-RIN	83	OCNE	567±28	594±27	-92	0.6
(10 male pairs)		CNE	336±33	346±33	11	0.9
		NE	221±28	230±28	1	1
		E	24±7	13±4	68	**0.068**
POST-RIS	80	OCNE	634±29	558±29	270	0.16
(8 male pairs)		CNE	347±33	286±30	262	0.19
		NE	259±31	200±26	245	0.2
		E	9±3	6±2	16	0.8
POST-(RIN+RIS)	163	OCNE	600±20	576±20	414	0.5
(18 male pairs)		CNE	342±23	317±22	506	0.3
		NE	240±21	215±19	481	0.3
		E	17±4	9±2	188	0.14

Table 2. Dominance odor recognition in POST fight phase: Results and Statistical evaluation. Dom /Sub: Female preference for Dominant vs Subordinate Males. Dom and Sub: mean time females spent on side of Dominant vs Subordinate male (in seconds ± SEM); H-Dom and SD-Dom: as above, but separated by pairs where Healthy male or Shell-Diseased male was dominant. (Note: In the RIN population all healthy males were dominant, so no SD-Dom category.) See also Table 1.

Dom/Sub	n	Flume area	Dom (s)	Sub (s)	W (D-S) (s)	p
POST-RIN	83	OCNE	567±28	594±27	-92	0.6
(10 male pairs)		CNE	336±33	346±33	-11	0.9
		NE	221±28	230±28	-1	1
		E	24±7	13±4	68	**0.068**
POST-RIS	80	OCNE	662±29	538±29	364	**0.059**
(8 male pairs)		CNE	371±36	269±28	223	0.3
		NE	279±33	186±24	239	0.2
		E	9±3	6±2	114	**0.031***
POST-(RIN+RIS)	163	OCNE	597±20	579±20	242	0.7
(18 male pairs)		CNE	337±23	321±22	183	0.7
		NE	239±21	216±19	210	0.7
		E	16±4	10±2	240	**0.056**
H-Dom						
POST-(RIN+RIS)	133	OCNE	609±22	561±22	283	0.5
(15 male pairs)		CNE	352±27	300±24	273	0.5
		NE	250±24	201±21	277	0.4
		E	20±5	9±2	176	**0.063**
POST-RIS	50	OCNE	651±41	536±42	139	0.17
(5 male pairs)		CNE	365±45	278±39	105	0.3
		NE	286±44	196±32	109	0.2
		E	9±4	6±2	26	0.4
SD-Dom						
POST-RIS	30	OCNE	594±37	606±37	-17	0.7
(3 male pairs)		CNE	289±51	312±50	-45	0.4
		NE	210±47	215±43	-39	0.4
		E	7±3	8±5	-6	0.6

surprising given that lobsters use odor for so many social situations. However, one can also conclude that the results are not surprising. Shell disease has been found in its current form only since 1997, a span of two lobster generations, a very short amount of time for any recognition and aversion to the disease to evolve. In addition, recognition of shell disease would not impact sexual selection if the disease were not spread through contact. Shell disease does affect the ability of male lobsters to establish dominance as healthy males were dominant in 15 of 18 fights ($p = 0.008$). This could be explained easily by the physiological changes found by Tarrant et al. (2012) reflected in low arginine kinase expression in muscle tissue. Since the crusher claw more than anything else determines the outcome of dominance fights, a weakened crusher muscle would indirectly affect male competitiveness and, thus, evolutionary fitness.

At first, it seemed surprising that females did not more strongly prefer dominant healthy males to shell diseased subordinate males. However, females did not recognize shell disease and their lack of significant preference for the dominant male has been seen earlier. Bushmann and Atema (2000) found that while pre-molt females significantly preferred dominant males ($p = 0.01$), inter-molt females showed less than significant preference for dominant males ($p = 0.07$). Our current study showed a similar trend favoring the dominant male in the criterion of entering attempts by RIN and RIS females ($W = 240$; $p = 0.056$; Table 2). Pre-molt females might have shown much clearer preference for dominant males as they did in large communal tanks ("mesocosms", (Cowan & Atema 1990) and in odor choice tests (Bushmann & Atema 2000). However, within our facilities and within the time frame available for this study it was impossible

for the lobstermen to obtain the number of healthy pre-molt females (in summer) simultaneous with the required number of healthy and shell diseased inter-molt males from each of the two study sites.

clear; they spent exactly the same amount of time in the odor areas of healthy and diseased males no matter where in the choice tank and no matter how we tried to identify individual female differences. This is expected from intermolt females and males from the same site. Even the small but significant preference of females to enter the dominant's shelter corresponds to earlier results with intermolt animals.

The other result, that shell-diseased males lose fights and have problems establishing dominance, is also robust and suggests indirect negative consequences for reproduction. In addition, shell diseased males molt more frequently than healthy males and it may take several post-molt months before their shell hardens sufficiently to start winning fights again (Cowan & Atema 1990). Thus molting as well as shell disease put lobsters further down in the dominance order. This suggests that shell diseased males have fewer mating opportunities. In a reduced population such as exists currently in SNE, this could lead to a scarcity of suitable males to provide the reproductive input needed to build and maintain a healthy population. This male effect compounds the direct loss of ovigerous females from the population caused by their long inter-molt, egg-bearing period that allows shell disease to flourish to a lethal level.

Highly contagious diseases have been shown to drive disease recognition mechanisms in spiny lobsters (Behringer et al. 2006). We did not identify an avoidance behavior in female intermolt lobsters in this research. We did identify an indirect effect

of shell disease: diseased males lose fights. Female recognition and preference for dominant males could put shell-diseased, and therefore likely subordinate males, at a mating disadvantage, thereby reducing the number of reproductive males and suppressing population growth.

A PHOTOGRAPHIC METHOD FOR LOBSTER MORPHOMETRY AIMED AT SITE DISCRIMINATION

Introduction

The phenotype of an organism can provide insight into its habitat and behavior. Morphometrics, the analysis of shape, applies multivariate methods to lengths, widths, and angles. Morphometric analyses using homologous landmarks can be used to identify correlations and differences between physical characters (Rohlf & Marcus 1993). (A "character" in this context is a linear measurement of a body part.) Morphometric differences can occur between species, populations, or individuals due to genetic variation, environmental effects, or sexual dimorphism. The American lobster, *Homarus americanus,* presents a suitable model for morphometric analysis because of the hard exoskeleton that covers its entire body. Additionally, prior research has shown morphometric differences between sites (Saila & Flowers 1969; Campbell & Mohn 1982; Cadrin 1995).

Knowing the structure and dynamics of populations is pertinent to both scientific and management objectives. Stock identification is of special concern in economically important species. A stock is generally defined as a self-sustaining group of conspecifics that respond similarly to environmental changes; although the definition differs depending on the framework of study (Begg et al. 1999; Campbell & Mohn 1982; MacLean & Evans 1981). Stocks can be differentiated by genetic composition (greater allelic variance between than within stocks) or by morphometric features in which case they are considered "phenotypic stocks" (Cadrin 2000; Swain & Foote 1999).

Morphometric features may differ due to genetic drift (Endler 1973), post-settlement selection (Hedgecock 1986), plastic responses to environmental differences (Pfennig et al. 2010), or a combination of factors (Palumbi 1994).

Past morphometric studies on H. americanus

The population of American lobster, *Homarus americanus*, in the Northwest Atlantic appears to consist of multiple phenotypic stocks, although the identities and boundaries of the stocks remain poorly defined. Based on relatively few morphometric characters, Perkins and Skud (1966) initially suggested that inshore and offshore lobsters could be differentiated based on characters including abdomen width, which may reflect differences in the maturity schedules of inshore and offshore lobsters. Abdomen width and claw size are secondary sex characters that become increasingly prominent at and after maturity. Saila and Flowers (1969) and Rogers et al. (1967) found morphometric differences between inshore and offshore adult and larval lobsters respectively. Campbell and Mohn (1982) saw morphometric differences between populations in the Bay of Fundy, the Scotian Shelf, and the Gulf of Maine and Cadrin (1995) found differences between inshore (Buzzards Bay) and offshore lobsters (Hydrographer's Canyon) based on secondary sex characters.

Digital measurement techniques

Lobsters are suitable subjects for repeatable body measurements because of the hard exoskeleton and defined body segments (Jolicoeur & Mosimann 1960). Digital

landmark techniques have been used to measure decapods in the past (Cadrin 1995; Hopkins & Thurman 2010; Rosenberg 2002). Cadrin (1995) utilized video recorded individuals to examine secondary sexual characters and allometric relationships, successfully discriminating between a sample of lobsters from an offshore canyon and another from an inshore bay south of Cape Cod. Based on 63 hand-measured characters of nearly all body parts, Radcliffe (2011) was able to discriminate between lobsters from 22 different sites currently under investigation in our lab.

In this study, we used the same 63 morphometric characters (Table 3) to compare the reliability, accuracy and resolution of photograph-based and hand-based measurements to discriminate between phenotypes of different sites. We also evaluated measurement time at the dock and observer training. To develop the method we used a sub-sample of three of our 22 capture sites, two nearby inshore sites ~15km apart and one relatively close offshore site ~100km to the SE (Fig. 3).

The goal of this project was to find an efficient and yet high-resolution method to compare lobster phenotypes within and between capture sites. In addition to improved discrimination, a successful method would create a permanent record for later verification, additional statistical analyses, and future comparisons with new samples. Thus, the specific objectives of this methodological study were to: 1) develop a photographic method of measuring lobsters that reduced sampling time, improved measurement reliability, and allowed for re-sampling, 2) compare the photographic and hand measurement methods for their ability to discriminate between lobsters from different sites.

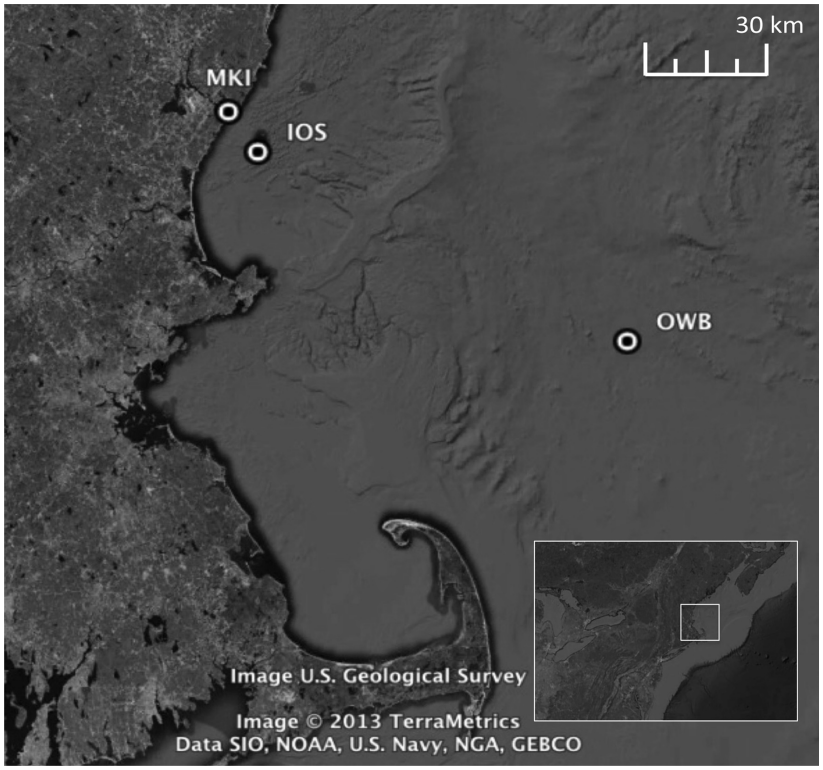


Figure 3. Map of sites for photographic measurement collection.

Methodology

The lobsters for this study were collected by commercial fishermen in August and September 2010 from three sites comprising areas of 1-10 km^2: Isle of Shoals (IOS) (15 females, 15 males), nearby Great Bay in Kittery (MKI) (18 females, 10 males), and offshore Wilkinson Basin (OWB) (20 females, 10 males) (Fig. 3). Lobsters were visually inspected upon landing: males and females were sexed using the sexually dimorphic anterior pleopod and then separated. Animals with damaged, regenerated or missing appendages as well as very large individuals were rejected. Accepted lobsters were ID-banded for proper identification of individuals. All photographs and hand measurements of the MKI lobsters were taken in the laboratory in Boston, whereas the photographs and hand measurements of OWB and IOS lobsters were taken indoors near the landing dock

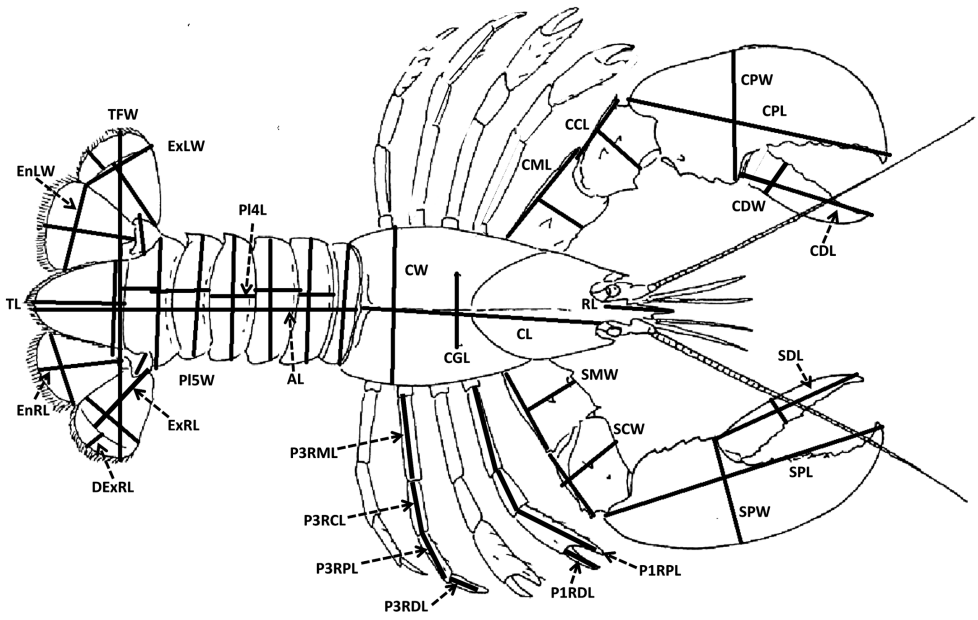


Figure 4. Diagram of dorsal measured characters.

immediately upon landing. In both methods, 63 morphometric characters were obtained (Fig. 4, Table 3).

Hand measurements were taken using digital calipers at a precision of 0.5mm. Following hand measurements, lobsters were quickly patted dry and harnessed to the dorsal restraint board (Appendix 1). Each lobster was positioned such that the carapace and abdomen were aligned and parallel with the board and the ventral side of the lobster was compressed to the board. The tail fan of the lobster was manually spread to maximum width. After three photographs were taken, the lobster was moved to the ventral restraint board for another three photos (Appendix 1). Chelae, pereopods, and the lower abdomen were strapped down with maximum extension. All photographs were captured using a Canon Powershot SD1400 with image stabilization at an optical zoom of 1.5x. The point of focus was automatically set for each shot. Light levels in the two measurement locations were similar and gave good photographic results. Calibration blocks were secured to the board at 6 different, regularly spaced heights. Each character

was paired with the calibration block closest to the same height. The calibration blocks were used to convert the photographic measurements from pixel-based lengths to millimeter-based lengths.

In a few cases body parts that, upon closer inspection, appeared regenerated or damaged were not measured and were counted as missing data. The same set of characters was measured in both techniques. Sexes were pooled together for regression analysis of each measurement. For all discriminant analyses, males and females were separated due to sexual dimorphism of mature lobsters (Templeman 1944; Saila & Flowers 1968).

Each digital image was measured using imageJ (Rasband 1997) by student observers trained in the photographic method. Each observer was provided with a template where each character measurement was displayed and briefly explained. TFW was eliminated from all analyses due to excessive measurement error. Additionally, abdomen length (AL) was removed from analysis because, in the photo method, the change in height between the posterior end of the carapace and the tip of the abdomen caused a small variance that could not be eliminated by a geometric correction factor. AL removal had no statistical impact on discrimination between sites. This left 61 characters for use in subsequent analyses.

We obtained estimates of *inter-observer* variance by having multiple observers use both methods to measure each lobster and *intra-observer* variance by having the same observer measure a training set of lobsters between 2 and 4 times using each measurement method. The mean, standard deviation, and range were calculated for each

character. Intra-observer variance was calculated by comparing like measurements (i.e. same lobster, same character) made by the same observers while inter-observer variance was calculated by comparing like measurements made by different observers.

Statistical Analysis

To compare the two measurement techniques we regressed the collected hand measurements against photo measurements for each character. Although the photographic method provides the ability to measure with a precision of 0.1mm, to perform a direct comparison to hand measurements, digital measurements were rounded to a precision of 0.5mm for regression and discriminant analyses.

To identify possible morphometric differences between sites, a series of linear discriminant function analyses (DFA) were performed using the statistical package JMP (Anon 2012). This analysis assigns individuals to capture sites based on similarities and differences in multivariate data so that some individuals are correctly assigned to the capture site while others can be "miss-assigned" to another site if their character set more closely resembles the morphometric pattern of the other site than its own capture site. For each DFA, lobsters were pooled by site and sex. First, the estimate of overall assignment rate between sites was calculated by repeating a linear discriminant function analysis 500 times with repeated random sub-sampling cross validation of the observed data (Solow 1990). Prior to each iteration, 5% of individuals were randomly selected from the entire data set and excluded from the training step of the DFA; those individuals were then used to validate the DFA.

Within each pool (i.e. individuals of the same sex from the same site), the site of origin was randomly reassigned. The number of individuals from each site was maintained during randomization. Using the randomized data set, the predicted assignment rate between sites was calculated by repeating the cross-validated DFA. Again, prior to each iteration, 5% of all individuals were randomly selected and excluded from the training step of the DFA; those individuals were then used to validate the DFA. After each set of 100 iterations, the sites of origin were randomly re-assigned. The p-values for site assignment rates were then estimated by comparing the assignment rate of individuals correctly assigned to their respective sampling site from randomized data with the observed rate: a significantly greater observed correct assignment rate indicates a morphometrically distinct set of lobsters.

Results

Comparison between measurement methods: regression results

Linear regression of matching hand and digital measurements from all individuals (n=88) indicated large differences in the levels of variance between characters: R^2-values varied from 0.17 (P1RCL) to 0.96 (Pl4W) (Table 3). Additionally, only 13 characters showed no statistical difference between methods based on slope and intercept values (Table 3). These characters were located on the carapace (CL, RL, CGL), abdomen and tail fan (Pl2L, Pl3L, Pl5l, PrRL), and both chelae (CMW, CCL, SIL, SIW, SMW, SCL). Characters of the carapace (CL, CW, RL, CGL), chela propodi (CPL, CPW, SPL, SPW), and pleonite widths (Pl1W – Pl6W) showed the least variance between measurement

Table 3. Comprehensive list of all 63 characters measured in both hand and photographic methods with a comparison of the two methods for 61 characters (n=88).

Character	Character code	Slope	SD	*F*	*p*	Intercept	SD	*F*	*p*	R^2
Carapace length	CL	0.99	0.04	0.25	0.80	−0.35	3.33	0.10	0.92	0.896
Carapace width	CW	0.89	0.03	3.63	<0.01	2.84	1.63	−1.74	0.09	0.909
Rostrum length	RL	0.97	0.05	0.49	0.62	2.09	1.60	−1.31	0.19	0.796
Cervical groove length	CGL	1.08	0.05	−1.64	0.11	−1.93	1.38	1.40	0.17	0.844
Abdomen length	AL*	—	—	—	—	—	—	—	—	—
Pleonite 1 width	Pl1W	0.90	0.03	3.69	<0.01	4.41	1.36	−3.25	<0.01	0.866
Pleonite 2 length	Pl2L	0.99	0.10	0.11	0.91	−2.58	1.58	1.63	0.11	0.547
Pleonite 2 width	Pl2W	0.89	0.03	3.31	<0.01	6.63	1.83	−3.63	<0.01	0.892
Pleonite 3 length	Pl3L	1.02	0.12	−0.16	0.87	−2.52	1.89	1.33	0.19	0.477
Pleonite 3 width	Pl3W	0.87	0.03	4.18	<0.01	7.75	1.68	−4.62	<0.01	0.905
Pleonite 4 length	Pl4L	0.67	0.11	3.11	0.00	2.33	1.82	−1.28	0.20	0.320
Pleonite 4 width	Pl4W	0.91	0.02	4.58	<0.01	3.92	1.05	−3.73	<0.01	0.963
Pleonite 5 length	Pl5L	1.01	0.20	−0.06	0.95	0.33	3.28	−0.10	0.92	0.237
Pleonite 5 width	Pl5W	0.89	0.02	5.05	<0.01	4.42	1.20	−3.67	<0.01	0.948
Pleonite 6 length	Pl6L	0.44	0.09	6.40	<0.01	9.24	1.87	−4.94	<0.01	0.215
Pleonite 6 width	Pl6W	0.83	0.03	6.11	<0.01	6.96	1.40	−4.96	<0.01	0.896
Tail fan width	TFW*	—	—	—	—	—	—	—	—	—
Exopod left length	ExLL	0.42	0.06	9.63	<0.01	14.34	1.58	−9.07	<0.01	0.378
Dexopod left length	DExLL	0.35	0.08	8.72	<0.01	7.07	0.92	−7.66	<0.01	0.210
Exopod left width	ExLW	0.52	0.05	9.01	<0.01	14.52	1.54	−9.41	<0.01	0.541
Endopod left length	EnLL	0.46	0.06	9.28	<0.01	16.35	1.67	−9.80	<0.01	0.436
Endopod left width	EnLW	0.48	0.06	9.29	<0.01	13.84	1.54	−9.00	<0.01	0.480
Protopod left length	PrLL	0.63	0.13	2.74	0.01	6.88	2.03	−3.39	<0.01	0.212
Telson length	TL	0.54	0.06	8.35	<0.01	8.18	1.92	−4.26	<0.01	0.516
Telson width	TW	0.54	0.06	7.16	<0.01	15.64	2.15	−7.27	<0.01	0.442
Protopod right length	PrRL	0.98	0.15	0.14	0.89	1.35	2.27	−0.60	0.55	0.339
Endopod right length	EnRL	0.71	0.05	5.65	<0.01	8.81	1.48	−5.95	<0.01	0.616
Endopod right width	EnRW	0.62	0.09	4.21	<0.01	9.19	2.48	−3.70	<0.01	0.611
Exopod right length	ExRL	0.42	0.06	9.63	<0.01	14.34	1.58	−9.07	<0.01	0.556
Dexopod right length	DExRL	0.67	0.08	3.86	<0.01	2.53	1.06	−2.39	0.02	0.434
Exopod right width	ExRW	0.60	0.06	6.74	<0.01	11.89	1.73	−6.87	<0.01	0.553
Crusher ischium length	CIL	0.65	0.06	5.41	<0.01	15.48	2.57	−6.03	<0.01	0.544
Crusher ischium width	CIW	0.73	0.11	2.52	0.01	3.87	2.65	−1.46	0.15	0.342
Crusher merus length	CML	0.80	0.07	2.73	0.01	6.36	3.87	−1.64	0.10	0.587
Crusher merus width	CMW	0.92	0.12	0.64	0.52	−3.93	3.32	1.18	0.24	0.409
Crusher carpus length	CCL	0.90	0.07	1.47	0.15	4.75	2.35	−2.02	0.05	0.691
Crusher carpus width	CCW	0.62	0.07	5.72	<0.01	8.88	2.02	−4.40	<0.01	0.516
Crusher propodus length	CPL	0.82	0.05	3.43	<0.01	19.92	6.08	−3.27	<0.01	0.737
Crusher propodus width	CPW	0.82	0.05	3.52	<0.01	8.34	2.66	−3.13	<0.01	0.757
Crusher dactyl length	CDL	0.83	0.07	2.56	0.01	10.61	3.66	−2.90	<0.01	0.653
Crusher dactyl width	CDW	0.56	0.08	5.33	<0.01	5.74	1.45	−3.96	<0.01	0.351
Seizer ischium length	SIL	0.99	0.07	0.16	0.88	1.30	2.67	−0.49	0.63	0.728
Seizer ischium width	SIW	0.87	0.09	1.53	0.13	−0.25	2.07	0.12	0.90	0.555
Seizer merus length	SML	0.70	0.07	4.37	<0.01	11.85	3.68	−3.22	0.00	0.565
Seizer merus width	SMW	0.90	0.10	0.97	0.34	−3.57	2.67	1.34	0.18	0.507
Seizer carpus length	SCL	0.95	0.06	0.88	0.38	3.19	2.22	−1.44	0.16	0.744
Sezier carpus width	SCW	0.74	0.07	3.97	<0.01	4.81	1.99	−2.42	0.02	0.609
Seizer propodus length	SPL	0.90	0.03	3.06	<0.01	9.45	4.11	−2.30	0.02	0.833
Sezier propodus width	SPW	0.88	0.05	2.60	0.01	4.01	2.05	−1.96	0.05	0.751
Seizer dactyl length	SDL	0.80	0.06	3.58	<0.01	11.94	3.76	−3.18	<0.01	0.686
Seizer dactyl width	SDW	0.71	0.13	2.34	0.02	4.20	1.72	−2.45	0.02	0.291
Pereopod 1 right ischium length	P1RIL	0.70	0.06	4.59	<0.01	7.60	1.81	−4.20	<0.01	0.579
Pereopod 1 right merus length	P1RML	0.54	0.06	7.85	<0.01	16.59	2.54	−6.53	<0.01	0.489
Pereopod 1 right carpus length	P1RCL	0.28	0.07	10.51	<0.01	11.68	1.62	−7.19	<0.01	0.170
Pereopod 1 right propodus length	P1RPL	0.51	0.07	7.13	<0.01	18.56	2.60	−7.14	<0.01	0.394
Pereopod 1 right dactyl length	P1RDL	0.60	0.09	4.53	<0.01	7.20	1.61	−4.47	<0.01	0.355
Pereopod right total length	P1RL†	0.54	0.05	10.07	<0.01	55.68	5.76	−9.67	<0.01	0.619
Pereopod 3 right ischium length	P3RIL	0.59	0.07	5.72	<0.01	11.10	1.89	−5.88	<0.01	0.443
Pereopod 3 right merus length	P3RML	0.46	0.07	7.46	<0.01	17.64	2.50	−7.04	<0.01	0.322
Pereopod 3 right carpus length	P3PCL	0.46	0.07	7.52	<0.01	7.18	1.53	−4.69	<0.01	0.330
Pereopod 3 right propodus length	P3RPL	0.61	0.06	6.36	<0.01	9.50	1.80	−5.27	<0.01	0.551
Pereopod 3 dactyl length	P3RDL	0.62	0.13	2.91	<0.01	6.19	2.09	−2.96	<0.01	0.217
Pereopod 3 total length	P3RL‡	0.65	0.04	8.12	<0.01	44.26	5.11	−8.66	<0.01	0.734

Note: Gray rows indicate the 13 characters where both slope and intercept values were not significantly different. *Eliminated from regression analysis. †P1RL in photographic method was calculated by summing P1RIL, P1RML, P1RCL, and P1RPL. ‡P3RL in photographic method was calculated by summing P3RIL, P3RML, P3RCL, P3RPL, and P3RDL.

techniques ($0.74<R^2<0.96$, Table 3). Selected regressions are shown in Fig. 5. Pleonite length measurements (Pl1L-Pl6L) showed a high degree of variance, as did measurements of several interior tail fan characters such as PrLL (Table 3). Of the 63 characters, 37 exhibited R^2 values greater than 0.5.

Compared to hand measurements, the photographic method greatly reduced both the intra- and inter-observer measuring variance (Table 4). This might be expected given that hand measurements are taken from a moving animal in the field while digital measurements are taken from a still image.

Discriminatory ability of both methods:

Both methods showed significantly higher correct assignment rates (i.e. assignment to the capture site) for the observed data than for the randomized data (Table 5). Overall, the photographic method outperformed the hand method in discriminatory ability for IOS and OWB females and for IOS, MKI, and OWB males while the hand

Table 4. Intra-observer and inter-observer error rates (mean+/- SD). Mean and standard deviation are calculated from error rates of all characters. Intra-observer variance was calculated by comparing like measurements (i.e. same lobster, same character) made by the same observers while inter-observer variance was calculated by comparing like measurements made by different observers.

	Hand		Photo	
	Range (mm)	Mean (mm)	Range (mm)	Mean (mm)
Intra-obs.	0–1.5	0.53±0.12	0.00–0.096	0.02±0.03
Inter-obs.	0–2.5	0.58±0.19	0.02–0.5	0.049±0.04

Note: Mean and standard deviation (SD) are calculated from error rates of all characters. Intra-observer variance was calculated by comparing like measurements (i.e., same lobster, same character) made by the same observers, while inter-observer variance was calculated by comparing like measurements made by different observers.

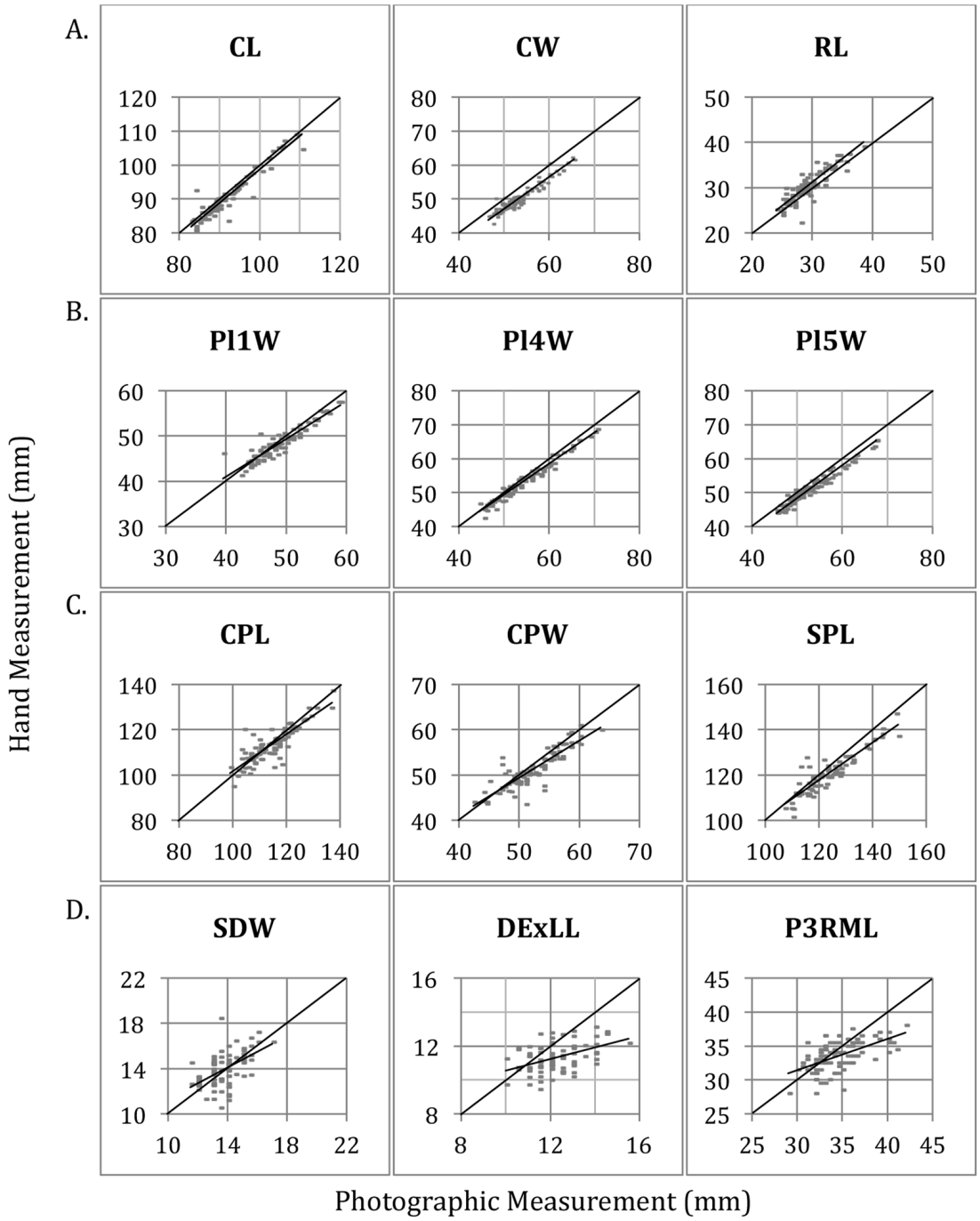


Figure 5. Comparisons of hand and photographic methods. Selected regressions of hand measurements versus the matching digital measurements (n=88). Row A: Non-sexually dimorphic traits. Row B: Female-associated sexually dimorphic traits. Row C: Male -associated sexually dimorphic traits. Row D. Traits with relatively poor agreement between hand and digital measurements. Linear regression y=mx+b; y = hand measurement, x = digital measurement.

method outperformed the photographic method for MKI females (Table 6). Miss-assignments of the observed data varied depending on the sex, capture site, and measurement method (Table 5). For example, IOS females that were measured by hand and miss-assigned were assigned evenly to MKI and OWB while MKI males that were measured by photograph were nearly always reassigned to IOS. Males and females for IOS and OWB showed similar assignment rates between hand and photographic methods. MKI females showed higher correct assignment rates than males with hand measurements while MKI males showed higher correct assignment rates than females with photographic measurements.

Discussion

The goal of this study was to find an efficient, reliable and yet high-resolution method to compare lobster phenotypes within and between capture sites. We developed a photographic measurement method and compared it to the previously used hand measurement method. The photographic method reduced sampling time, improved measurement precision, and allowed for re-sampling; it also resulted in improved discrimination capabilities. Regression analysis showed that some characters exhibited good correspondence between methods (i.e. measured similarly in both methods) while others were significantly different (Table 3; Fig. 5). However, the inter- and intra-observer variances were significantly improved with the photographic method (Table 4). Both the hand and the photographic methods showed that lobsters from the three sites

Table 5. Average discrimination results. Top: randomized data: lobsters were randomly reassigned to a site of origin before running the cross-validated DFA (n=500). Bottom: observed data (n=500).

		Assignment (%)					
		Female			Male		
Method	Location	IOS	MKI	OWB	IOS	MKI	OWB
Randomized data (*n* = 500)							
Hand	IOS	31.1±2.8	30.9±4.7	39.0±5.4	24.2±4.7	36.4±3.5	39.4±5.3
	MKI	40.0±5.2	23.9±5.1	36.1±4.2	32.1±4.3	27.1±5.8	40.8±3.7
	OWB	34.0±4.9	28.5±6.4	37.4±5.2	39.3±6.3	33.4±6.2	27.3±4.0
Photo	IOS	27.6±3.9	40.4±3.1	32.0±4.5	31.4±4.0	29.9±3.6	38.6±2.7
	MKI	37.7±4.6	36.8±3.6	25.4±4.4	36.6±7.1	46.9±6.3	16.4±4.1
	OWB	39.3±6.3	33.4±6.2	27.3±4.0	30.0±4.9	32.2±4.1	37.8±6.1
Observed data (*n* = 500)							
Hand	IOS	47.6±5.1	28.3±3.8	24.1±4.2	54.1±4.2	23.6±3.7	22.3±4.4
	MKI	26.3±6.3	72.6±6.6	1.1±0.1	13.5±4.5	58.2±4.0	28.3±4.8
	OWB	14.2±2.3	23.1±3.6	62.7±3.9	18.1±4.2	23.3±3.8	58.6±4.7
Photo	IOS	60.4±5.5	24.9±5.2	14.8±4.2	66.0±5.1	17.9±4.2	16.1±3.4
	MKI	23.0±2.7	57.7±3.1	19.3±2.8	22.6±4.3	73.3±4.5	4.2±1.8
	OWB	12.2±3.1	15.1±3.6	72.7±5.3	8.3±4.9	21.9±4.5	69.9±5.1

Note: For the randomized data, lobsters were randomly reassigned to a site of origin before running the cross-validated discriminant function analyses.

examined were morphometrically dissimilar; however, the photographic method was generally superior to the hand method (Table 6). With the exception of MKI, males and females were discriminated equally within each measurement technique, suggesting the absence of sex-based site differences. While the discrimination of observed MKI individuals was always significantly greater than the randomized data, hand measurements showed greater correct assignment of MKI females while the photographic measurements showed greater correct assignment of MKI males. This difference could be, in part, furthered by the difference in measurement error between the two methods.

Many characters that contributed significantly to site discrimination were located on body parts not associated with the obvious secondary sex characters (Templeman 1935; Saila & Flowers 1969). While past studies have utilized mainly secondary sex characters to differentiate between lobsters from different sites (Perkins and Skud 1966,

Table 6. Direct comparison of correct random and observed assignments as found using both hand and photographic methods. P-values represent proportion of assignment rates from the random data set that were higher than assignment rates from the observed data set. Observed results show significantly greater correct assignment than random indicating significant morphometric site differences.

	% Correctly assigned (±SD)					
	IOS		MKI		OWB	
	Hand	Photo	Hand	Photo	Hand	Photo
Female (*n* = 500)						
Random	31.1±2.8	27.6±3.9	23.8±5.1	36.8±3.6	37.4±5.2	27.3±4.0
Observed	47.6±5.2	60.4±5.5	72.6±6.7	57.7±3.1	62.7±3.9	72.7±5.3
p	0.004	<0.002	<0.002	<0.002	<0.002	<0.002
Male (*n* = 500)						
Random	24.2±4.7	31.4±4.0	27.1±5.8	46.9±6.3	27.3±4.0	37.8±6.1
Observed	54.1±4.2	66.0±5.1	58.2±4.0	73.3±4.5	58.6±4.7	69.9±5.1
p	<0.002	<0.002	<0.002	<0.002	<0.002	<0.002

Note: *p* values represent proportion of assignment rates from the random data set that were higher than assignment rates from the observed data set. Observed results show significantly greater correct assignment than random results, indicating significant morphometric site differences.

Saila & Flowers 1969; Cadrin 1995), our results suggest that there are many non-sexual measurements of potential importance to site discrimination. Since the most discriminant characters also differed between males and females and between hand and photographic measurement techniques (Table 5), it appears necessary to measure many characters to confidently identify characters that successfully discriminate between lobsters from different sites. The ideal of a few universal discrimination characters did not materialize within this study; however, sampling additional sites might provide a better understanding of universal measurements.

A total of 13 characters showed non-significant differences between hand and photographic measurements. These characters were mostly located on the carapace, abdomen, and major claws. High coefficients of determination indicated that there was little variance between hand and photographic methods that was not accounted for by a linear regression (Table 3). The characters most comparable between the two methods

($0.74<R^2<0.96$) include those on the carapace (CL, CW, RL, CGL), chelae propodi (CPL, CPW, SPL, SPW), pleonite widths (Pl1W – Pl6W), and total pereopod lengths (P1RL, P3RL). Characters that showed no significant differences between hand and photographic measurements or had high R^2 typically had distinct and easily visualized points of origin. Although a number of characters (i.e. CL, RL, CGL, etc.) appeared more than once in the lists of "15 most discriminant" (Table 7), most did not, showing that a universal "best character" list did not emerge.

Both intra- and inter-observer variance were improved significantly using the photographic method. Hand calipers are only precise to 0.5mm while digital measurements can be precise to approximately 0.1mm. To compare the two methods directly, we rounded the digital measurements to the nearest 0.5mm. While the photographic method has the ability to be more precise, lobsters that exhibited darker carapaces were the most difficult to measure digitally and often showed slightly greater variance in dorsal measurements. Conversely, individuals with lighter carapace coloration were easier to measure digitally and exhibited small variance in those measurements.

While both measuring methods were effective in site discrimination and showed significantly higher correct assignment to capture sites than predicted from random assignments, the photographic method often performed somewhat better than the hand method (Table 5, 7). It would be inappropriate, however, to speculate that the observed phenotypic differentiation of these sites makes them stable stocks because for this

Table 7. The 15 most discriminant measurements by sex and measurement type as determined by stepwise selection (in decreasing order of discriminant contribution). Note that the same characters (e.g. CGL) appear at different contribution ranks and thus contribute somewhat differently to the overall population discrimination

	Male		Female	
Character	Hand Rank	Photo Rank	Hand Rank	Photo Rank
CL	10	4	14	4
CW	6	1	1	1
RL			8	9
CGL		10	12	2
Pl1W	4	5		8
Pl2L		3	7	3
Pl2W				
Pl3L	7	2		
Pl3W				
Pl4L	5	6	5	14
Pl4W	13	13		7
Pl5L		8	11	5
Pl5W	11	9		12
Pl6L				
Pl6W				11
ExLL				
DExLL	8			
ExLW				
EnLL			2	
EnLW				
PrLL	15			
TL	2			
TW	3		4	
PrRL				13
EnRL	1	14	3	
EnRW		15		
ExRL				
DExRL				
ExRW				
CIL				
CIW				
CML	12		13	
CMW				
CCL				10
CCW				
CPL				
CPW				
CDL		12		
CDW				
SIL				6
SIW		7		
SML				
SMW		11		
SCL				
SCW				
SPL				
SPW				
SDL				
SDW				
P1RIL			15	15
P1RML			6	
P1RCL				
P1RPL			9	
P1RDL				
P1RL			10	
P3RIL				
P3RML				
P3PCL				
P3RPL	9			
P3RDL				
P3RL	14			

Note: Note that the same characters (e.g., CGL) appear at different contribution ranks and thus contribute somewhat differently to the overall population discrimination. Refer to Table 1 for description of characters.

evaluation of measurement techniques we used only three capture sites and we did not evaluate temporal stability of lobster site morphometry

Practical considerations

The photographic-digital method has several important advantages over the hand measuring method. Hand measurements take approximately 7-10 minutes per individual with two researchers and approximately 10-12 minutes per individual with one researcher (Table 8). In contrast, at the dock, the photographic method takes approximately 4 minutes per individual for one researcher; the speed of data collection facilitates field measurement and/or increased sample size. In addition, with hand measurements, lobsters

are typically measured once and training for the technique requires extensive effort to ensure fair replication across researchers. Also, outlier data points emerging during analysis cannot be verified and have to be removed from the data set. Importantly, lobster images can be re-measured repeatedly within the lab once the photograph has been captured. Training and assessment of observer bias are efficient because both can be done in the lab with a specific training set of images and assessed separately. Once trained, measurement time takes approximately 8 minutes per individual. Also, because the

Table 8. Benefits of the photographic method

Scientific	Applied
Ability to resample and cross-validate	Minimal observer training
Increased precision of measurements	Reduced time at dock
Increased discriminatory ability	
Opportunity to perform blind studies	
Test additional hypotheses	

photographs are in high resolution and cover the entirety of the dorsal and ventral sides of each lobster, they provide the opportunity for testing additional hypotheses using any number of software platforms.

Overall, the photographic method described here provides a viable technique to measure lobsters and has improved ability to discriminate between spatial (and temporal) samples. The technique has several practical advantages and appears to have promise for future field application due to decreased sampling time and effort and relative ease of observer training. Further sampling is required to ensure that this technique will be effective for measuring individuals below 80mm CL and above 120mm CL.

GENETIC STRUCTURE AMONG POPULATIONS OF *H. americanus* THAT DISPLAY MORPHOMETRIC DIFFERENCES

Introduction

Identifying and delineating management units and/or stock structure for commercially exploited fisheries is a major challenge for modern conservation biology. Accurately defining stock boundaries is critical because intense fishing pressure can have dramatic impacts on stock health and, in the event of a fishery collapse, the resulting recovery (Ciannelli et al. 2013). However, population structure in marine systems is often characterized by relatively high levels of gene flow and large effective population sizes resulting in low levels of genetic differentiation, complicating the identification of stock structure (Domingues et al. 2010; Knutsen et al. 2011; Hess et al. 2012; Teacher et al. 2013; Corander et al. 2013; Benestan et al. 2015).

Previous efforts to characterize population structure in marine organisms have revealed high levels of connectivity with low, but statistically significant structure, in species as diverse as Atlantic cod (average F_{ST} = 0.0037, (Knutsen et al. 2011)), Pacific lamprey (average F_{ST} = 0.010, (Hess et al. 2012)), Atlantic Herring (average F_{ST}= 0.008, (Teacher et al. 2013)), and copper rockfish (average F_{ST}=0.031, (Dick et al. 2014)). Similar patterns have also been observed in multiple species of reef fish (Purcell et al. 2006; Priest et al. 2012; D'Aloia et al. 2014), mollusks (Piggott et al. 2008), and crustaceans (Domingues et al. 2010; Gslason et al. 2013). Although the significance of weak population structure has been debated (Knutsen et al. 2011), several studies have also found evidence for morphological and behavioral divergence within marine species

despite low levels of genetic differentiation (Knutsen et al. 2011; Hess & Narum 2011; Hess et al. 2012; Corander et al. 2013), including the American lobster (Rycroft et al, in prep; Benestan et al. 2015)). This is especially significant because the American lobster is a commercially important species, contributing to the economies of both Canada and the United States, therefore the potential management implications of genetic structure within this species is both biologically and economically relevant (Campbell & Mohn 1982; Fogarty 1995; Benestan et al. 2015).

The American lobster has high dispersal potential at both larval and adult life-stages. Hatching occurs between May and September (Ennis 1995) and the pelagic larvae take 11-54 days before molting into the final larval stage and then 2-6 days before settling behaviors begin (Cobb et al. 1989). During the pelagic larval period, lobsters have the potential for long distance transport (Cobb et al. 1989; Katz et al. 1994; Ennis 1995; Incze & Naimie 2000; Harding et al. 2005). In addition to larval dispersal, adult lobsters are also capable of long distance movements as tagging studies have shown some adult lobsters to undertake long distance migrations (Saila & Flowers 1968; Cooper & Uzmann 1971; Campbell 1986; Campbell 1989; Campbell & Stasko 1986; Estrella & Morrissey 1997; Bowlby et al. 2007; Bowlby et al. 2008; Scopel et al. 2009). Adult lobsters have also shown potential homing abilities by returning to the same locations after long migrations (Pezzack & Duggan 1986; Pezzack & Duggan 1986). Bowlby et al. (2007; 2008) found that individual lobsters differed in their migration behaviors as some acted as “residents” and others as “dispersers.” This was also observed at a small scale (Karnofsky et al. 1989). Additionally, individual animal migrations may or may not

affect genetic population structure as it is unknown whether there is gene flow associated with them.

Early population structure studies on the American lobster using allozyme markers (Tracey et al. 1975) and RAPD analyses (Harding et al. 1997) showed effectively no population structure among regions although several recent studies using microsatellite (Crivello et al. 2005; Kenchington et al. 2009; Rycroft et al. in prep) and RADseq (Benestan et al. 2015) techniques have shown weak, but statistically significant, population structure. Both Kenchington et al. (2009) and Benestan et al. (2015) identified the greatest level of genetic differences between southern (i.e. Gulf of Maine and Rhode Island) and northern samples (i.e. Gulf of St. Lawrence) while relative pairwise differences within each region were lower. These results were interpreted as a possible signature of post-glacial expansion (Kenchington et al. 2009) or the existence of an oceanographic barrier to larval dispersal (Benestan et al. 2015), however both of these studies focused on berried (i.e. egg bearing) females.

Rycroft et al. (*In prep*) identified significant morphological differences between groups of male and female lobsters separated by distances <50km suggesting the possibility of greater genetic structure than identified in prior studies. Here, to address this issue, and to examine patterns of genetic connectivity between morphometrically dissimilar groups of lobsters (*H. americanus*), we analyzed population genetic structure among morphologically dissimilar groups of lobsters with genome-wide SNP markers via RADseq.

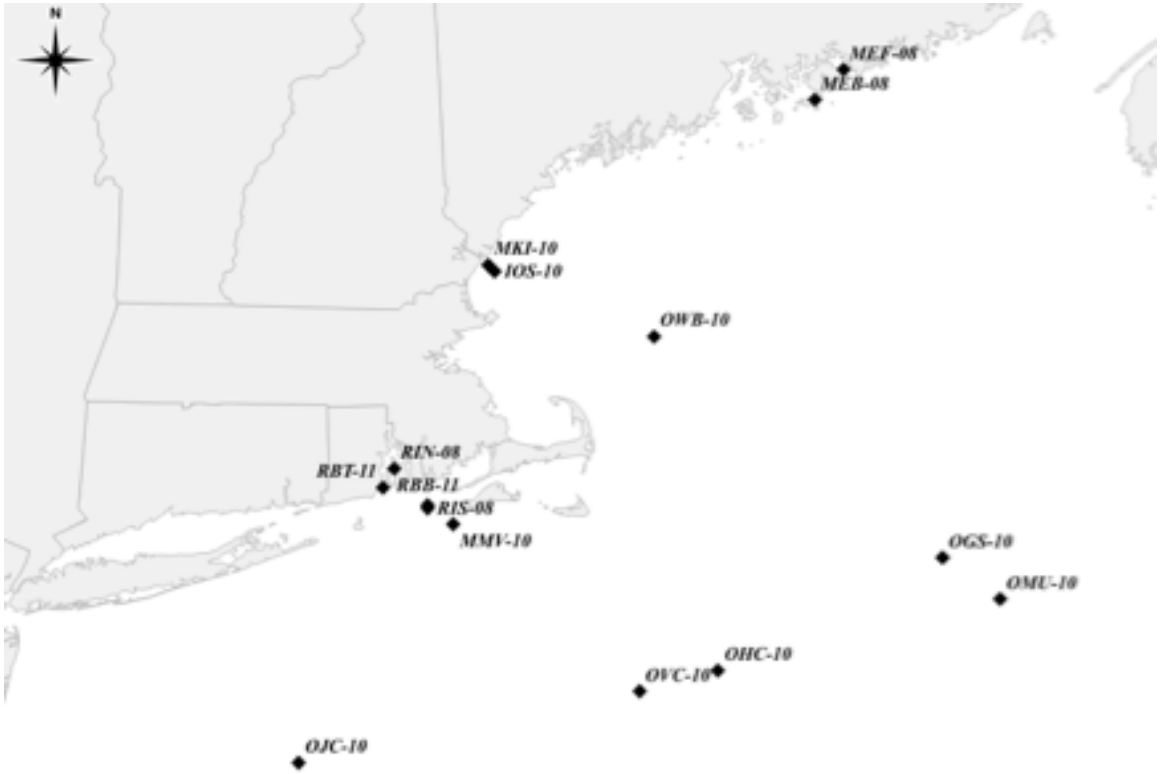


Figure 6. Sites sampled for RADseq analysis.

Methodology

Sampling

Lobsters were collected by commercial fishermen from 15 sites including 9 inshore sites and 6 offshore sites (Fig. 5). Offshore sites ranged from the Hudson Canyon in the south to Wilkinson Basin in the north. Sites in Maine (MEB and MEF) and New Hampshire (MKI and IOS) were collected from sites separated by approximately 30km. Sites in Rhode Island (MMV, RBB, RBT, RIN, and RIS) were clustered within and just

Table 9. Description of sampling locations: sample date, latitude, longitude and number of individuals successfully genotyped (N_{GEN})

SITES	LOCATION	SAMPLE DATE	Lattitude	Longitude	N_{GEN}
MEF	Frenchmans Bay	2008	44.385000°	-68.171389°	19
MEB	Blue Hill Bay	2008	44.172778°	-68.372500°	20
MKI	Kittery	2010	43.011944°	-70.669722°	20
RIN	Narragansett Bay	2008	41.573333°	-71.328056°	17
RIS	Rhode Island Sound	2008	41.298333°	-71.089722°	17
MMV	Marthas Vineyard	2010	41.186389°	-70.912778°	17
IOS	Isle of Shoals	2010	42.969167°	-70.617500°	19
OHC	Hydrographers canyon	2010	40.150000°	-69.050000°	17
OWB	Wilkinsons Basin	2010	42.508056°	-69.503889°	19
OGS	Georges Basin	2010	40.943056°	-67.475278°	10
OVC	Veatch canyon	2010	40.000833°	-69.606944°	16
OMU	Munson canyon	2010	40.651389°	-67.067500°	9
OJC	Jones canyon	2010	39.500000°	-72.000833°	12
RBB	Brown's Bank	2011	41.322500°	-71.092500°	18
RBT	Beavertail	2011	41.441111°	-71.408889°	18

outside of Narragansett Bay. Offshore sites were selected in consultation with the Atlantic Offshore Lobstermen's Association (AOLA). It was attempted to gather 20 males and 20 females at or greater than first legal carapace length from each site, however, due to sex ratios, it was not always possible to collect the full 20 of each sex. For tissue samples, a pleopod was removed from each individual and stored in 95% EtOH for tissue samples. A total of 288 lobsters (15 sampling sites with, on average, 19.2 lobsters per site) were successfully sequenced (Table 9).

DNA extraction and Library Preparation

Genomic DNA was extracted using Qiagen Blood and Tissue kits following the kit protocol. DNA quality was confirmed using visual inspection on 1% agarose gel followed by quantification with Quantit Picogreen dsDNA assay kits. RAD-sequencing

libraries were prepared following a protocol developed on American lobster by Benestan *et al.* (2015). Each individual was barcoded with a unique six-nucleotide sequence and 48 individuals were pooled per lane. Real-time PCR was used to quantify libraries. Single read, 100 bp target length, sequencing on Illumina HiSeq2000 platform was conducted at the Genome Quebec Innovation Centre (McGill University, Montreal, Canada).

Bioinformatics and genotyping

The libraries were demultiplexed using the *process_radtags* program in stacks v.1.19 (Catchen et al. 2013). Raw sequencing data was checked in FASTQC (Andrews 2010). Reads were truncated to 80 bp and adapters sequences were removed with CUTADAPT to obtain reads with the same length. The formation of RAD loci was allowed with a maximum of three nucleotide mismatches (M = 3), according to Ilut *et al*. (2014) and a minimum stacks depth of three (m = 3), among reads with potentially variable sequences (*ustacks* module in stacks, with default parameters*)*. Then, reads were aligned *de novo* with each other to create a catalogue of putative RAD tags (*cstacks* module in stacks, with default parameters). In the *populations* module of stacks and following consecutive filtering steps, we first retained SNPs genotyped in at least 70% of the individuals found in at least 9 of the 15 sampling sites (Table 10). Potential homologs were excluded by removing markers showing heterozygosity > 0.50 (Hohenlohe et al. 2011) and $F_{IS} < 0.30$ and $F_{IS} > -0.30$ within samples. A minor allele frequency of greater than 5 per cent was selected for the analysis. The resulting filtered VCF files were converted into the file formats necessary for the following analyses using PGDspider v.2.0.5.0 (Lischer & Excoffier 2012).

Table 10. Counts of remaining putative loci after filtering steps and final numbers of SNPs and loci.

FROM READS TO SNPS	**SNP count**	**Loci count**
STACKS CATALOG	119,811	26371
POPULATION FILTERS		
Genotyped		
> 80% of the samples	26,544	5,935
> 80% of the populations		
MAF FILTERS		
Local MAF > 0.02	4,148	2,737
Local MAF > 0.2		
COVERAGE FILTER		
From 10 to 100x	4,075	2,685
HWE FILTERS		
Hardy-Weinberg equilibrium		
(P-value 0.05)	2,553	2,110
FIS between -0.3 and 0.3	1,869	1,484
$H_{OBS} < 0.5$	1,767	1,424
Linkage Desequilibrium < 0.8	1,717	1,422
Sex-linked markers	1,705	1,411

Population differentiation

We quantified the extent of pairwise population differentiation using the unbiased F_{ST} estimator q (Weir & Cockerham 1984) in the software GENODIVE v2.0b27 (Meirmans & Van Tienderen 2004). Significance of the observed F_{ST} values was determined by running 10,000 permutations. We used the function *hclust* available in the R package *ggdendro* to create a UPGMA dendrogram based on the F_{ST} values. A heatmap was produced to illustrate the F_{ST} matrix considering four different F_{ST} groups delimited from the distribution of pairwise F_{ST} values. In addition, we conducted a standard Mantel tests to correlate genetic distances (F_{ST}) and natural logarithm of Euclidian geographical distances. The Mantel test included was performed on all pairwise comparisons with GENODIVE and significances of the tests were assessed using 20,000 permutations.

Population clustering

We inferred population structure by using two Bayesian clustering methods that are implemented in the programs FASTSTRUCTURE v2.3.4 (Raj et al. 2014) and admixture v1.23 (Alexander et al. 2009). Both programs provide a means of identifying the best value for *K*, the number of putative populations. With FASTSTRUCTURE, we used 10,000 burn-in iterations followed by another 10,000 Markov chain Monte Carlo (MCMC) steps assuming an admixture model based on individuals and including no prior information on sampling location. We ran admixture using 20,000 bootstraps. For both programs, we varied the number of groups (*K*) from 1 to 16 with 5 iterations for each value and stabilization of parameters was checked for this length of burn-in and MCMC. We then performed a Discriminant Analysis of Principal Components (DAPC) in the R package *adegenet* (Jombart & Ahmed 2011). The DAPC is a non-model-based method, which maximizes differences between groups while minimizing variation within groups. We first evaluated the optimal number of discriminant functions (n=60) to retain according to the optimal α-score obtained from our data (Jombart & Ahmed 2011). In addition, a *K-means* clustering analysis was performed on sampling locations with the Genodive v.2.0b25 program (Meirmans & Van Tienderen 2004), using simulated annealing and testing for *K* clusters from 1 to 10, for 5000 permutations. This analysis provides the Calinski-Harabasz pseudo-*F*-statistic for determining the number of clusters (Caliński & Harabasz 1974).

Results

Genotyping results

The average number of sequence reads among the 8 libraries was 172 million (range: 158-187 million) and the average number of quality filtered reads per library was 130 million (range: 87-156 million), providing a total of 119,811 SNPs. After applying the different filtering steps, 1705 SNPs out of the 119,811 SNPs were retained for subsequent analyses (Table 10). From these, we removed 112 individuals (~31.1%) that showed more than 30 percent of missing data at these SNPs. Of the 1705 SNPs retained for subsequent analyses, the missing data across individuals per SNP was low with, on average, 9.7% of loci with missing data (range: 1.6 to 31.8%).

Selecting candidate SNPs for demographic inference

From the 1705 SNPs retained, ARLEQUIN removed 327 SNPs (~19.1%) from the outlier detection analysis, due to the missing data. Of the 1378 SNPs analyzed by ARLEQUIN, a genome scan detected 1280 SNPs seemingly not under selection (~75.1%), 67 SNPs (~3.9%) under divergent selection and 31 SNPs (~10.9%) potentially under balancing selection. The F_{ST} obtained for each SNP with ARLEQUIN ranged from -0.0273 to 0.1002 and averaged 0.0112.

With 10,000 prior odds, BAYESCAN identified 1312 SNPs (~76.9%) seemingly not affected by selection and 393 SNPs (~23.1%) potentially under balancing selection. In this case, BAYESCAN did not detect any SNPs potentially under divergent selection. Using 10 prior odds, BAYESCAN detected 9 SNPs potentially under divergent selection

with a P-value < 0.05. On the other hand, considering the Jeffreys'scale for Bayes factors of evidence lead us to identify a total of 21 SNPs potentially under selection: 9 SNPs substantial, 5 SNPs strong, 3 SNPs very strong and 4 SNPs decisive evidence for the selection (Fig. 7). Subsequent inferences of genetic structure were carried out using the 1614 SNPs (~94.6%) candidate neutral markers identified by BAYESCAN and ARLEQUIN.

Population structure

All lobsters analyzed were grouped into a single cluster according to FASTSTRUCTURE and the DAPC using 1614 potentially neutral SNPs. The same result was obtained when we included all 1705 SNPs (results not shown). The *K-means* analyses revealed an optimal *K* of one cluster, according to the lowest BIC and the pseudo-*F*-statistics. Thus, all the sampling locations were assigned as being one homogenous genetic population.

The F_{ST} comparison results were congruent with the DAPC and FASTSTRUCTURE. Overall, only two of the 105 pairwise comparisons of genetic differentiation between sampling locations, IOS vs. RIN and IOS vs MMV, were significant (P-value < 0.01) and none were significant after Bonferroni correction. When replacing the 38 negative F_{ST} values by zero, the average F_{ST} was 0.0014 across the 1614 SNPs and all pairwise comparisons of the 15 sampling sites with maximum of 0.0047

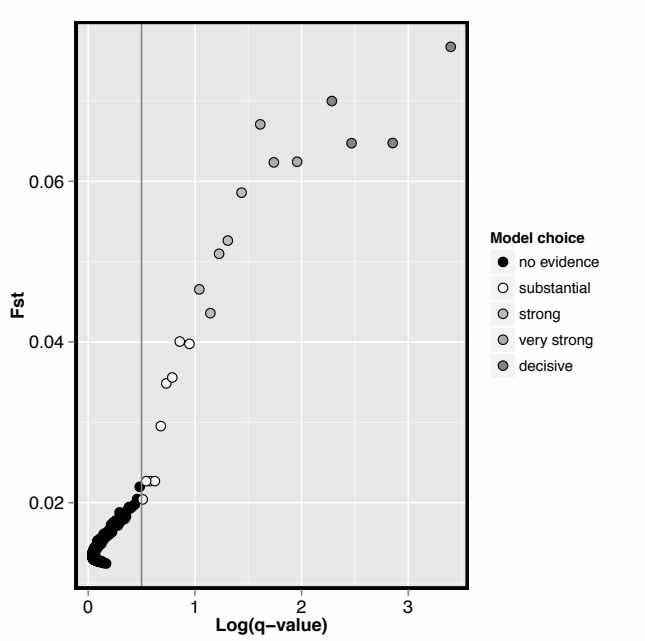


Figure 7. SNPs identified by Bayescan as putatively under selection.

(MMV vs. IOS) (Table 11). Both the heatmap and the dendrogram based on F_{ST} values showed no clear clustering of the samples (Fig. 8). We found no evidence for isolation by distance effect (r^2= -0.08, P-value = 0.74) when considering all pairwise comparisons and all SNPs (Fig. 9).

Discussion

The purpose of this study was to determine whether genetic differences existed among groups of lobsters that have been found to display site-based morphological differences. Although prior findings of genetic differences among populations of lobsters using various genetic techniques have shown results ranging from low but significant structure to essentially no structure (Tracey et al. 1975; Harding et al. 1997; Crivello et al. 2005; Kenchington et al. 2009; Benestan et al. 2015), the significant morphological differences found by Rycroft et al. (2013) and Rycroft et al. (In Prep) suggested the potential for greater genetic structure amongst these populations.

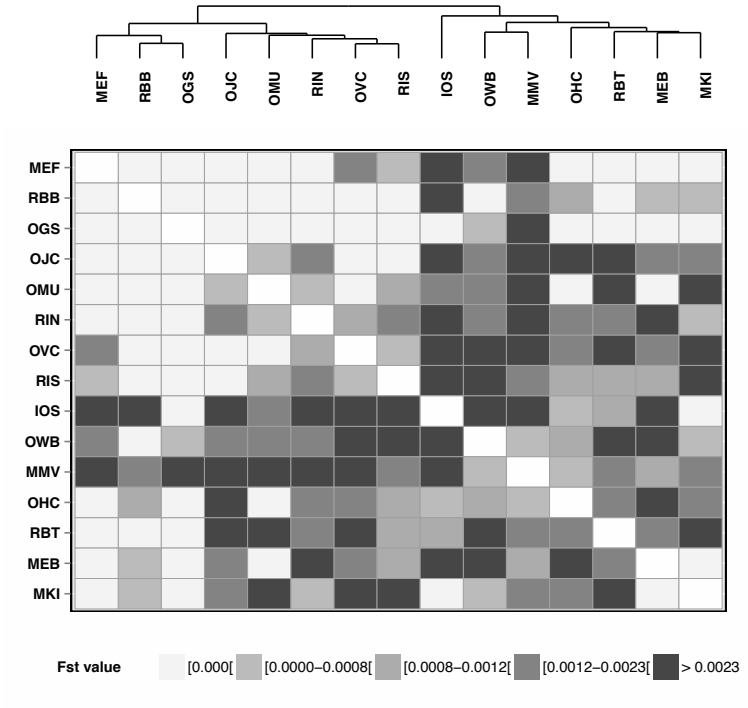


Figure 8. Heatmap and UPGMA dendogram based on F_{ST} values obtained by analysis of putatively neutral SNP loci.

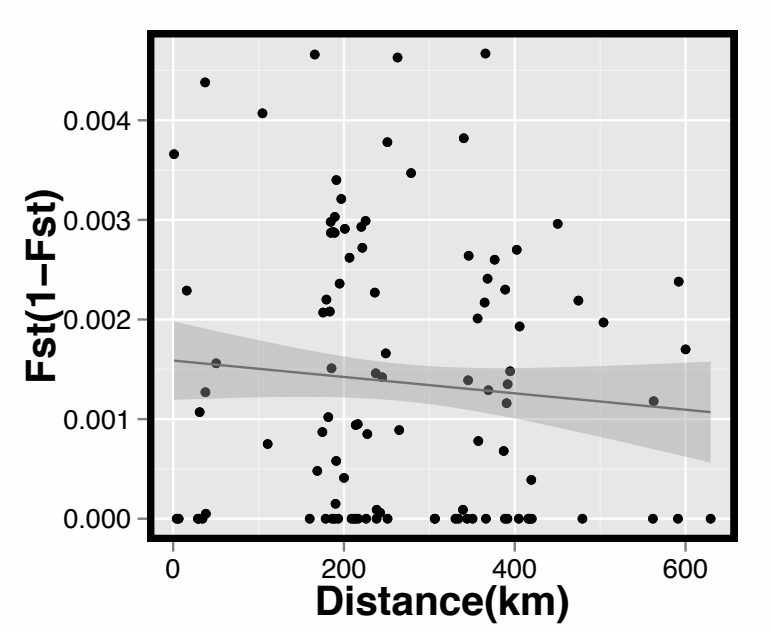


Figure 9. Isolation by distance based on pairwise F_{ST} values of neutral markers and

Table 11. Pairwise F_{ST} values for the 1614 putatively neutral SNPs (top) and associated p-values (bottom). Grey cells indicate significant ($p<0.01$) F_{ST} values.

Statistic	OVC10	OWB	RBB	RBT	RIN08	RIS08	IOS	MEB	MEF	MKI	MMV	OGS	OHC	OJC	OMU
OVC10	--	0.00347	0	0.00293	0.00085	0.00015	0.00382	0.00219	0.00197	0.00264	0.00287	0	0.00156	0	0
OWB	0.10538	--	0	0.00321	0.00208	0.00303	0.00407	0.00262	0.00227	0.00075	0.00078	0.00006	0.00089	0.00148	0.00201
RBB	0.69046	0.53629	--	0	0	0	0.00288	0.00068	0	0.00058	0.0022	0	0.00095	0	0
RBT	0.08238	0.08998	0.90982	--	0.00229	0.00107	0.00102	0.00135	0	0.00287	0.00151	0	0.00142	0.00272	0.00298
RIN08	0.34053	0.22076	0.5107	0.10458	--	0.00127	0.00466	0.0026	0	0.00048	0.00291	0	0.00166	0.00146	0.00041
RIS08	0.46831	0.13437	0.83983	0.28014	0.23795	--	0.0034	0.00116	0.00039	0.00236	0.00207	0	0.00094	0	0.00087
IOS	0.03679	0.06199	0.05399	0.27874	0.0076	0.04839	--	0.00299	0.00378	0	0.00467	0	0.00009	0.0027	0.00217
MEB	0.12078	0.13217	0.34633	0.19276	0.07159	0.26415	0.09378	--	0	0	0.00118	0	0.00296	0.0017	0
MEF	0.14877	0.17497	0.76165	0.91642	0.56129	0.41792	0.0214	0.91642	--	0	0.00238	0	0	0	0
MKI	0.07099	0.36353	0.34633	0.03479	0.37972	0.09298	0.77864	0.64727	0.56689	--	0.00129	0	0.00139	0.00193	0.00241
MMV	0.08958	0.38952	0.11658	0.19196	0.06979	0.14957	0.0094	0.25875	0.09778	0.21696	--	0.0023	0.00009	0.00438	0.00366
OGS	0.84823	0.4793	0.96981	0.75985	0.80304	0.53629	0.86323	0.71686	0.95021	0.78064	0.20656	--	0	0	0
OHC	0.21396	0.37572	0.30454	0.23275	0.20396	0.33153	0.47231	0.06019	0.68146	0.21476	0.4753	0.75585	--	0.00463	0
OJC	0.77325	0.35513	0.90622	0.13977	0.28974	0.55909	0.15317	0.22835	0.87203	0.19576	0.05759	0.86863	0.05099	--	0.00005
OMU	0.82404	0.35053	0.4943	0.16097	0.44131	0.39312	0.25735	0.60408	0.80604	0.18956	0.12937	0.86323	0.59568	0.4979	--

Results of the current study indicate that there is limited to no significant genetic population structure at putatively neutral SNP loci among these morphometrically different samples of individuals. It is possible that with greater sample sizes, statistically significant F_{ST} values would appear; however, the high levels of gene flow between regions would likely not change as shown by Benestan et al. (2015) utilizing identical genotyping methods with larger sample sizes and an average F_{ST} of 0.0019 compared to the present findings of 0.0014. Additionally, Benestan et al. found that, within the Gulf of Maine and Rhode Island, there were very small genetic differences (average F_{ST}= 0.0015) suggesting that a larger sample size in this study might have seen more statistically significant results. Nonetheless, the value of the F_{ST} statistics from this study are in line with prior genetic studies on *H. americanus* and suggest that the source of the morphometric differences is mostly not due to neutral genetic divergence but, instead, due to either plastic responses to environmental differences or local adaptation at or near morphology-determining loci in the genome (Berglund & Lagercrantz 1983; Kenchington & Glass 1998; West-Eberhard 2003; Stapley et al. 2010; Hess et al. 2014).

The finding of small levels population structure and high levels of gene flow is a common and growing refrain in studies of marine organisms where low but statistically significant levels of genetic differentiation have been identified in a number of marine species (Hess et al. 2012; Knutsen et al. 2011; Zemeckis et al. 2014; André et al. 2011; Priest et al. 2012; D'Aloia et al. 2014), including a number of crustaceans including the shore crab (Silva et al. 2010), European spiny lobster (Babbucci et al. 2010), and swimming crab (Weber & Levy 2000) among others. These relatively low, but

significant, F_{ST} values were also seen in recent studies on *H. americanus* as well (Kenchington et al. 2009; Benestan et al. 2015). Both the Kenchington and Benestan studies showed small but significant differences between northern lobsters and southern lobsters with greater differences between the north and south than within either region. Additionally, both studies studied egg-bearing females which could have increased the observed genetic differences. Within this study, we only sampled lobsters from the southern region (i.e. south of Nova Scotia) and included males and non-egg bearing females.

The results of FASTSTRUCTURE, DAPC, and K-means analyses support our findings of little to no significant genetic structure, as each analysis identified only a single cluster of individuals, suggesting that all individuals were a part of the same genetically similar group. Each technique has different capabilities of identifying genetic structure at low F_{ST} values even with thousands of genetic markers (described in Benestan et al. 2015) with DAPC showing the greatest efficiency at detecting clustering in populations with weak ($F_{ST}<0.01$) genetic differences. The lack of any clustering beyond a single group, however, is a strong indication of the absence of neutral genetic structure.

Examination of the heat map and dendrogram (Fig. 8) created from F_{ST} values shows no identifiable geographic pattern of genetic differentiation and the Mantel test showed no signal of isolation by distance. With non-significant and seemingly random genetic differences, the SNP data might seem to contradict those prior findings of Benestan et al. (2015) and Kenchington et al. (2009); however, because of the increased

range of sampling, larger sample sizes, and the use of only ovigerous females in those studies, it is difficult to directly compare these findings. As such, it is not necessarily prudent to discuss management implications based upon this study's SNP data alone. Additionally, a microsatellite analysis run in parallel to this SNP analysis (Rycroft et al., *In prep*) showed small but significant genetic structure.

In sum, it is likely that weak neutral genetic population structure in *H. americanus* exists across its range with the greatest genetic differences existing between individuals in the Gulf of Maine and the Gulf of St. Lawrence and an additional area of differentiation suggested south of Cape Cod. For the purposes of informing management decisions and to truly resolve these genetic differences and to establish temporal stability, it will be necessary to conduct at multi-year study sampling many individuals of varying sizes, sexes, and egg-bearing conditions from the same sites each year. Additionally, the sampling of larvae, juveniles, and adults of varying sizes would provide a greater detail of whether genetic structure exists and, if so, whether that structure originates at settlement or at some later developmental stage.

Although our SNP results do not show significant neutral genetic structure among populations of lobsters in the Gulf of Maine, Offshore, and Rhode Island regions, even in the presence of weak genetic structure, the existence of morphometric structure between these sites suggests one of two hypotheses; plastic responses to environmental variables that differ between sites or local adaptation to selective pressures that cause genetic differences at relatively small regions of the genome not analyzed in this study. Those hypotheses will be explored in Chapter 4 of this dissertation.

WHAT IS THE DRIVER OF SITE-SPECIFIC MORPHOLOGICAL DIFFERENCES IN *H. americanus*? – IDENTIFYING CANDIDATE LOCI FOR LOCAL ADAPTATION

Introduction

Genetic analyses show that the American lobster (*Homarus americanus*) has low levels of genetic population structure while morphometric analyses suggest much higher levels of structure. As such, the morphometric differences found in *H. americanus* are likely due either to phenotypic plasticity, to local adaptation to selective pressures, or to a combination of both. Because the lobster population is so widely distributed, from the Carolinas to Laborador and from inshore to the continental shelf, local populations experience significantly different environmental conditions. It is due to these localized environmental differences that either variable plastic responses or selection of differential phenotypes could occur should variations in phenotype confer a fitness benefit (Kawecki & Ebert 2004; West-Eberhard 2005). As such, the American lobster is a suitable model for testing hypotheses as to the genetic or plastic origin of these morphometric differences using genome-wide analysis of SNP markers found using techniques such as RADseq (Nosil et al. 2009; Vincent et al. 2013).

Phenotypic Plasticity

Phenotypic plasticity is a common driver of individual differences in genetically similar organisms due to environmental effects. Plasticity can lead to behavioral, physiological, and anatomical differences that might be temporary or can become fixed

(West-Eberhard 2003). Crustaceans have displayed plastic variances in morphology, behavior, life history, and physiology due to environmental effectors including predation, prey, and abiotic factors (reviewed in Padilla & Savedo 2013). Additionally, plasticity has been shown in larvae (Ebert 1994; Davis et al. 2005), juveniles (Duarte et al. 2013) and adults (Twombly & Tisch 2000; Harrison et al. 2001; Marchinko 2003; Delgado & Defeo 2008; Edgell & Rochette 2009; Chandrapavan et al. 2010) of multiple crustacean species. It is likely that the ability to molt the hard exoskeleton and the ability to regenerate entire limbs provide crustaceans with a functional mechanism of remaining phenotypically flexible, responding to environmental changes with plastic responses (Pereira et al. 2014). Similar results have been seen in other species of crustaceans where morphological variation has existed with the apparent absence of genetic differentiation (Spivak & Schubart 2003; Brian et al. 2006; Haye et al. 2010; Jarrett 2008). It is thus possible, that lobsters could show a plastic behavioral or morphological response, in the form of behavioral or morphometric changes, to locally specific environmental factors.

Adaptive Variation/Local Adaptation

Local adaptation occurs when natural selection due to environmental variables force differential changes in allele frequencies . These selective pressures can impact individual loci or, more likely, a number of loci across the genome in a quantifiable manner. Through genome-wide association studies, it is possible to identify areas of the genome that are putatively under selection and to correlate these regions with specific morphometric, behavioral, or physiological differences, even in non-model organisms

(Hohenlohe et al. 2010; Goetz et al. 2010; Hess et al. 2014). *H. americanus* exhibits both great population size and high dispersal and, as a result, weak genetic population structure (Tracey et al. 1975; Kornfield & Moran 1989; Harding et al. 1997; Crivello et al. 2005; Kenchington et al. 2009; Benestan et al. 2015). This means that adaptive markers can be more easily identified through a genome scan approach than in a species with stronger population structure (Pérez-Figueroa et al. 2010; Hess et al. 2014). In the marine environment, local adaptation has been suggested as a potential driver of morphological and behavioral (i.e. timing of migrations) differences in organisms with high levels of neutral gene flow (Hecht et al. 2013; Hess et al. 2014). In this species, selection can occur pre-zygotic due to differential mating, or post-zygotic during the larval, juvenile, or adult life stages.

Pre-zygotic processes

Plasticity and selection can initially occur pre-zygotically, during the mate selection process. Lobsters are capable of individual recognition with males fighting with other males for both shelter and mating opportunities while females actively choose their mates (Bushmann & Atema 1997; Bushmann & Atema 2000; Karavanich & Atema 1998; Johnson & Atema 2005). Additionally, in flume experiments, females have shown the preference for the odors of males from their own region over the odors of males from other locations (Rycroft et al., In prep.). Whether the odor differences are a result of genetic variation or a plastic physiological difference due to dissimilar habitats is, as of yet, unresolved. It is also unknown whether those female-choice behaviors result in

positive assortative mating in situ. If so, it could provide a potential mechanism by which females can choose mates and drive selection of location-specific markers.

Post-zygotic processes

Once mated, females extrude and carry fertilized eggs attached externally to the ventral surface of the abodomen attached to the pleopods for approximately one year (Waddy et al. 1995). During this time, females are capable of long distance movement and, appear to move further and faster than females without eggs or males with the general movement offshore during the winter months and back towards shallower, and warmer, waters during the spring and summer, the general time of egg extrusion (Estrella & Morrissey 1997). Whether females return to the same area each time that they release their eggs is currently unknown although several studies have shown migrating lobsters to return to their original capture sites (Saila & Flowers 1968; Pezzack & Duggan 1986). If females do return to the same locations each time they release their eggs, it is possible, through stereotyped hydrodynamic and development processes, that offspring will recruit to locations within the release area or slightly downstream (Incze & Naimie 2000; Incze et al. 2010).

Plastic and selective responses to variable environments could also occur during and after the larval settlement process occurs. Settlement behavior is mediated through olfactory, physical, and visual cues (Botero & Atema 1982; Cobb et al. 1989; Boudreau et al. 1992; Lillis & Snelgrove 2010) and there is at least some behavioral plasticity in settlement time based on the suitability of benthic habitat (Botero & Atema 1982).

Settling lobsters specifically seek structurally complex substrates (i.e. cobble) with a preference for areas with macroalgae and relatively low light levels (Botero & Atema 1982; Johns & Mann 1987; Boudreau et al. 1990). Settling lobsters will move vertically throughout the settlement period to putatively test the benthic habitat and, as a result, delay their settlement and return to the pelagic environment more frequently in less desirable regions (Cobb et al. 1983). These decisions have direct implications for survival as larvae settled in more complex environments experience significantly lower levels of predation (Wahle & Steneck 1991; Palma et al. 1998; Palma et al. 1999). The settlement process results in a long-duration cryptic juvenile stage during which lobsters grow, leaving the shelter only after a period of growth and only at night, potentially due to the need to increase the amount of food they can obtain (Lawton 1987; Cobb & Wahle 1994). Due to variability in settler density and quantity of high quality benthic habitat across the lobster's range, differences in selective pressures are likely, potentially leading to the differential selection of variable adaptive traits or differential plastic responses in different locations.

This study seeks to identify candidate loci correlated with morphometric characters in lobsters. Additionally we test whether the morphometric differences that result from selective pressures on specific regions of the genome that are not resolved using putatively neutral markers in past population structure studies. To test this hypothesis, we completed a genome scan of lobsters that displayed significant morphometric differences at a small-scale regional level (Rycroft et al., In prep.). These

lobsters showed very low levels of neutral genetic differentiation based on a population structure analysis (Rycroft et al., In prep.).

Methodology

Note: For sampling, DNA extraction, library preparation, bioinformatics, and genotyping methodology, please refer to the methods section of Chapter 4.

Detecting SNPs under selection

We detected SNPs potentially under selection by using BAYESCAN v.2.1 (Foll & Gaggiotti 2008) as well as the F_{dist} approach (Beaumont & Nichols 1996) implemented in ARLEQUIN v.3.5 (Excoffier & Lischer 2010). Bayescan estimates population-specific F_{ST} coefficients by the Bayesian method described in Beaumont and Balding (2004). SNPs with a posterior probability over 0.95 were considered as outliers, after running 200,000 iterations on all samples together. We performed two Bayescan runs using different 'prior' odds values (10 and 10,000). The prior odds in these analyses represent the probability that the locus is under selection and, therefore, the prior odds used in this analysis assume that there are 10 and 10,000 times more neutral loci than loci that are under selection (Lotterhos & Whitlock 2014). For the first run, we used the more conservative prior odds (10,000) and then used the more permissive odds (10) for the second run. ARLEQUIN was executed with 200,000 simulations and 100 demes simulated as recommended by the authors, and SNPs were considered as outliers based on their F_{ST} and P-value.

Morphometrics-genomics association analysis

Sexual character and 63 hand-measured characters of nearly all body parts and of each lobster were collected during the sampling following the method developed in Rycroft et al. (In Prep). We ran the Latent Factor Mixed Models (LFMM) program on the first three principal components obtained from a multivariate Principal Component Analysis (PCA) of the morphometric data. LFMM is a method based on hierarchical Bayesian mixed model, which tested genotypic-phenotypic correlations based on the residuals of the principal component analysis while controlling for background population structure (Frichot et al. 2013). To account for population structure, we ran the analysis with the 1 *k* genetic group detected by the clustering methods described in Chapter 4. We ran the analysis 3 times per variable and kept all SNPs with a z-score > 3. We then applied a false discovery rate (FDR) correction of 5% on the *p*-values (Benjamini & Hochberg 1995) output by the LFMM program using the *qvalue* package (Storey 2002) in R (R Core Team 2013).

We also searched for association between SNPs and morphometry using TASSEL. To identify loci having significant associations with the 63 morphometric characters measured on each individual, we performed two different analyses using TASSEL 5.2.10 (Bradbury et al. 2007). A general linearized model (GLM) and mixed linearized model (MLM) were utilized to identify significant correlations between phenotypic characters and genotypes while controlling for population structure using a principal component analysis of putatively neutral SNPs (Hess et al. 2014). The SNP

PCA was created using the 1614 putatively neutral SNPs identified in Chapter 4, and the first three axes were included as covariates to represent population structure. In addition to accounting for population structure, the MLM also utilizes a kinship matrix calculated in TASSEL using the "scaled IBS" method to control for the effects of relatedness (Endelman & Jannink 2012). The kinship matrix was calculated using all 1717 SNPs identified in Chapter 4. MLM analysis was completed with "no compression" and using the "P3D (estimate once)" variance component estimation technique to group each individual separately while reducing the overall computation time (Zhang et al. 2010). GLM and MLM analyses were run with both raw character measurements and a 4-axis PCA on morphometry calculated by TASSEL. The PCA on morphometric characters was utilized to control for covariance of characters and the first four PC axes were selected for analysis (Hess et al. 2014; Cadrin 2000). Associations were calculated for each SNP against each of 63 morphometric characters and PC axes. GLM and MLM analyses were run through 10,000 permutations to calculate P-values.

Functional annotation

To identify functional genes in the SNPs of interest detected by both genome scan and environmental association approaches, we first performed a BLAST of these SNPs against the complete transcriptome of the American lobster (Spencer, *personal communication*). Then, we detected contiguous segments of DNA (contigs) containing SNPs of interest. These contigs, are longer than the 80bp RAD loci and were used to execute a BLAST analysis in the NCBI database. Blast-screens were performed using

liberal quality criteria (E-value < 1.0E-6; similarity > 70%) on all the 1,411 sequences and the few sequences of interest detected by genome scan and environmental association approaches.

Results

Note: For results pertaining to genotyping and outlier analyses, refer to Chapter 4.

Morphometric analyses linked to LFMM and TASSEL

Of all 1705 SNPs included in the association analyses, several markers stood out due to the sheer number of associations, specifically to sexually dimorphic characters. An examination of allele frequencies at these markers showed a dichotomy between males and females including 8 where females showed one unique allele while males displayed two alleles thus suggesting that these alleles were located on a sex chromosome. We will address this at length in a future manuscript as it is not within the scope of this manuscript. For further association analyses, these markers were removed due to their inherent correlation with sexually dimorphic characters in the claws and abdomen.

The first, the second and the third principal components (PCs) calculated through LFMM accounted for 62.9%, 9.4% and 6.9% of the total variation in lobster morphometry. In this PC analysis, the first PC axis was most heavily loaded on carapace tail-fan, and leg measurements (CL, TFW, TW, P1RML, P1RPL, and P1RCL). The second PC axis was positively loaded with claw measurements (top 18 loadings were characters on the crusher or seizer claws), and PC3 was largely representative of tail-fan, abdomen, and leg characters (ExLL, ExRL, P1RL, Pl1W). Based on a zscore > 3, the

program LFMM identified a total of 31, 4 and 11 SNPs significantly correlated with the first, the second and the third PCs of the morphometric data, respectively (Appendix 2). Only one SNP was found linked to both PC1 and PC2.

The first, second, third, and fourth PCs calculated through TASSEL accounted for 57.8%, 13.7%, 9.4%, and 7.8% of the total variation in morphometry. PC1 of the TASSEL analysis was largely dependent on leg length characters (P1RL and P3RL). PC2 was most heavily loaded with carapace width (CW). PC3 was heavily loaded on leg length (P1RL and P3RL) and negatively loaded on abdomen length (AL). PC4 was most heavily loaded on abdomen length and width (AL, Pl2W, Pl3W, Pl4W, and PL5W). The GLM identified 0, 41, 5, and 10 SNPs significantly associated with PC1, PC2, PC3, and PC4 with six markers significantly associated with two PC axes (Appendix 1). The MLM identified 38 markers significantly associated with PC1-PC4 with one marker significantly associated with two PC axes. Only one marker appeared in both the LFMM and TASSEL analysis and no markers were seen across all four techniques.

A TASSEL analysis testing for associations with raw character lengths identified 71 putatively non-sex linked loci with significant associations with at least one morphometric character. In the TASSEL GLM and MLM, there were a total of 69 and 47 markers, respectively, with significant associations with at least one morphometric character. Only two markers were found to significantly correlate with multiple

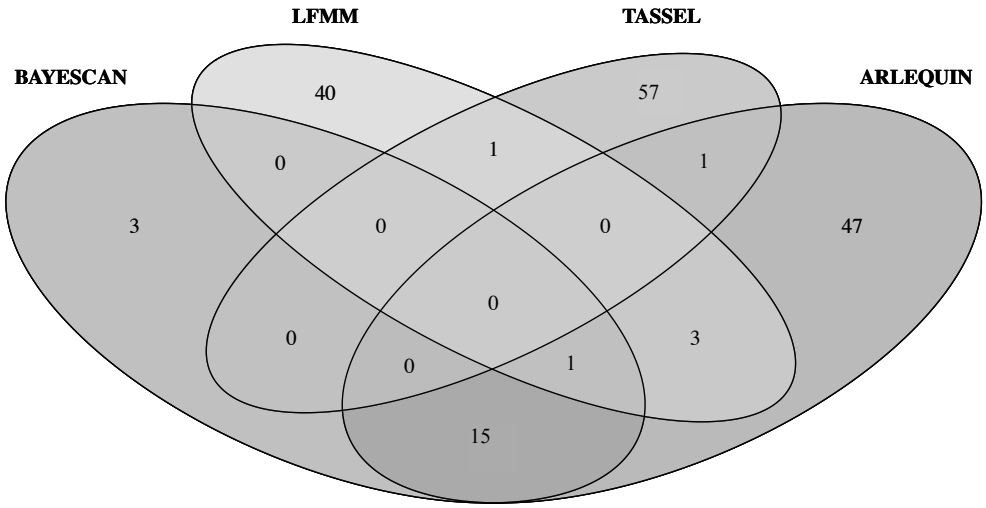


Figure 10. Venn diagram of number of markers found to be putatively under divergent selection through Bayescan and ARLEQUIN analyses and markers with significant associations to at least one PC factor score. TASSEL values include markers identified in either MLM or GLM analyses.

characters in the GLM and only one within the MLM (Table 12). Conversely, 36 markers were found by the GLM to significantly associate with carapace width and 12 significantly associated with the length of the 6th pleonite, which is the length of the final abdominal segment prior to the tail-fan (Table 13).

Comparison of the approaches and SNP candidate annotation

Using BLAST alignment tool on all the 1,411 RAD sequences, we detected 615 RAD sequences (43.5%) matching to the American lobster transcriptome. BLAST results were conservative, with an average of 91.9% sequences similarity (ranging between 79.4% and 100%) and maximum e-values of $3.00*10^{-7}$. The contigs containing the 615 RAD sequences ranged in length from 203 to 16207 nucleotides, and were then used to create the annotation.

Table 12. Number of morphometric characters significantly associated with each marker based on TASSEL analysis

Marker	MLM	GLM
10465	0	19
24252	0	1
171491	0	18
287004	1	1
329593	1	1
382228	0	1
463671	1	1
474876	1	1
505260	1	1
526286	1	1
581890	1	1
599026	1	1
633351	1	1
727552	1	1
755792	1	1
763655	1	1
784937	1	1
1001832	1	1
1026713	1	1
1058826	1	1
1090744	1	1
1108695	1	1
1235018	1	1
1387200	1	1
1409295	1	1
1409826	1	1
1481120	0	1
1494974	1	1
1644682	1	1
1655636	1	1
1680144	1	1
1758143	1	1

Marker	MLM	GLM
1770875	1	1
1845303	1	1
1909130	0	1
1963022	1	1
1989171	1	0
2009735	1	1
2061854	1	1
2082066	1	0
2119705	1	1
2151856	0	3
2152022	1	1
2283383	1	1
2319089	1	1
2504766	1	1
2516260	0	14
2539103	0	12
2544220	0	2
2597057	0	1
2669880	0	3
2761342	0	2
2841034	0	1
2862095	0	1
3031017	0	1
3042637	0	1
3161361	0	1
3201335	0	1
3304302	0	1
3661314	0	1
3754910	3	20
3755092	0	1
3815156	0	1
3879142	0	1

Table 13. Number of markers significantly associated with each morphometric character based on TASSEL analysis.

Character	MLM	GLM
CL	0	0
CW	29	38
RL	0	2
AL	0	5
Pl1W	1	1
Pl2L	0	4
Pl2W	3	12
PL3L	0	3
Pl3W	3	12
Pl4L	1	5
Pl4W	4	10
Pl5L	0	0
Pl5W	1	8
Pl6L	12	15
Pl6W	0	7
TFW	0	0
ExLL	1	4
DExLL	0	0
ExLW	0	0
EnLL	1	1
EnLW	0	0
PrLL	0	1
TL	0	0
TW	0	0
PrRL	0	0
EnRL	0	2
EnRW	0	0
ExRL	0	2
DExRL	0	1
ExRW	0	0
CIL	0	1
CIW	0	5

Character	MLM	GLM
CML	0	0
CMW	0	5
CCL	0	7
CCW	0	7
CPL	0	3
CPW	1	7
CDL	0	0
CDW	0	1
SIL	0	1
SIW	0	2
SML	0	1
SMW	0	5
SCL	1	6
SCW	0	8
SPL	0	1
SPW	0	1
SDL	0	0
SDW	0	1
CGL	0	8
P1RIL	0	1
P1RML	0	0
P1RCL	0	2
P1RPL	0	0
P1RDL	0	1
P1RL	0	0
P3RIL	0	0
P3RML	0	0
P3RCL	0	0
P3RPL	0	0
P3RDL	0	0
P3RL	0	0

From the 171 sequences of interest detected by LFMM, TASSEL, and outlier analyses, 15 sequences were located within a short distance of an annotated coding region (Table 14). Only one of these 15 sequences was detected by LFMM and outlier analyses. This sequence encodes a 40S ribosomal protein S3-A, which plays an essential role in cell proliferation, growth, and death in humans (Naora et al. 1998).

Discussion

The goal of this study was to identify candidate loci, identified through ARLEQUIN and Bayescan analyses, that correlate with morphometric differences between lobsters from various sites using two platforms, LFMM and TASSEL. During this study, we identified many sequences that were significantly associated with morphology and a number more that were putatively under divergent selection. This suggests the existence of differential local adaptation across the sampling sites, and a potential connection to the morphometric differences that have been identified between lobsters at different sites.

Identification of markers associated with morphology

TASSEL and LFMM analyses are designed to identify associations of SNP markers to environmental or morphometric traits (Bradbury et al. 2007; Frichot et al. 2013). These analyses were run in parallel in this study to maximize the number of candidate loci identified. While both TASSEL and LFMM identified a number of SNPs significantly associated with morphology, due to the finding of many markers

Table 14. A selection and characterization of high quality ($e<3*10^{-7}$) BLAST matches found by comparing *H. americanus* RAD-seq reads to various species against other species

Analysis	Locus	Contig Name	Uniprot	% Similarity	E-value	Species	Protein	Function
LFMM	2614	comp187389_c0_seq2	Q9M571	100	9.00E-19	Spinacia oleracea (Spinach)	Phosphoethanolamine N-methyltransferase	Catalyzes N-methylation of the three methylation steps required to convert phosphoethanolamine to phosphocholine. Mediates a key step in the biosynthesis of choline, a precursor of the osmoprotectant glycine betaine.
	36847	comp188318_c1_seq21	P42284	93.1	1.00E-28	Drosophila melanogaster (Fruit fly)	Longitudinals lacking protein	Putative transcription factor required for axon growth and guidance in the central and peripheral nervous systems.
	37647	**comp193322_c1_seq7**	**P02350**	100	4.00E-17	Homo sapiens (Human)	40S ribosomal protein S3-A	Involvement of ribosomal proteins in regulating cell growth and division and apoptosis
	45201	comp195436_c0_seq18	Q8IZ41	100	5.00E-41	Homo sapiens (Human)	Ras and EF-hand domain-containing protein	Intracellular protein transport. Binds predominantly GDP, and also GTP.
BAYESCAN	37647	**comp193322_c1_seq7**	**P02350**	100	4.00E-17	Homo sapiens (Human)	40S ribosomal protein S3-A	Involvement of ribosomal proteins in regulating cell growth and apoptosis
	31690	**comp193529_c1_seq11**	**Q24325**	100	0.00E+00	Drosophila melanogaster (Fruit fly)	Transcription initiation factor TFIID subunit 2	Mediates promoter responses to various activators and repressors. Functions in neurogenesis.
	7097, 55326	comp195858_c1_seq21	P35072	92.75, 92.77	2.00E-16	*Caenorhabditis briggsae* (Nematode)	Transposable element Tcb1 transposase	Probably essential for transposable element Tcb1 transposition. The insertion of Tcb1 is the main cause of spontaneous mutations.
	47035	**comp197967_c0_seq3**	**Q6ZQH8**	98.72	6.00E-47	Mus musculus (Mouse)	Nucleoporin NUP188 homolog	May function as a component of the nuclear pore complex (NPC)
ARLEQUIN	37647	**comp193322_c1_seq7**	**P02350**	100	4.00E-17	Homo sapiens (Human)	40S ribosomal protein S3-A	Involvement of ribosomal proteins in regulating cell growth and apoptosis
	31690	**comp193529_c1_seq11**	**Q24325**	100	0	Drosophila melanogaster (Fruit fly)	Transcription initiation factor TFIID subunit 2	Mediates promoter responses to various activators and repressors.
	4580	comp195292_c0_seq3	Q03172	100	3.00E-40	Mus musculus (Mouse)	Zinc finger protein 40	Transcription factor which binds specifically to the palindromic sequence 5'-GGGAAATCCC-3' in the alpha-A crystallin promoter.
	9816	comp195526_c0_seq5	Q3UV71	100	1.00E-115	Mus musculus (Mouse)	Transmembrane and TPR repeat-containing protein 1	Unknown
	47035	**comp197967_c0_seq3**	**Q6ZQH8**	98.72	6.00E-47	Mus musculus (Mouse)	Nucleoporin NUP188 homolog	May function as a component of the nuclear pore complex (NPC). Potentially involved in the regulation of HSF1-mediated heat shock response.
	8317	comp198502_c0_seq10	P27657	100	3.00E-82	Rattus norvegicus (Rat)	Pancreatic triacylglycerol lipase	Plays an important role in fat metabolism.
	33218	comp198791_c0_seq1	P11369	86.84	9.00E-16	Mus musculus (Mouse)	LINE-1 retrotransposable element ORF2 protein	DNA Recombination. Has a reverse transcriptase activity required for target-primed reverse transcription of the LINE-1 element mRNA, a crucial step in LINE-1 retrotransposition.
TASSEL	21977	comp195860_c0_seq162	Q868Z9	100	1.00E-166	Drosophila melanogaster (Fruit fly)	Papilin	Essential extracellular matrix (ECM) protein that influences cell rearrangements.
	17340	comp198451_c0_seq39	P05090	100	6.00E-27	Homo sapiens (Human)	Apolipoprotein D	APOD occurs in the macromolecular complex with lecithin-cholesterol acyltransferase. Probably involved in the transport and binding of bilin, a color binding protein.
	29272	comp199025_c0_seq1	Q7ZW46	98.51	2.00E-69	Danio rerio (Zebrafish)	UDP-xylose and UDP-N-acetylglucosamine transporter	Sugar transporter that specifically mediates the transport of UDP-xylose (UDP-Xyl) and UDP-N-acetylglucosamine (UDP-GlcNAc) from cytosol into Golgi.
	7917, 15148, 25074	comp199154_c0_seq2	Q9NBX4	91.78, 91.14, 93.67	2.00E-16	Drosophila melanogaster (Fruit fly)	Probable RNA-directed DNA polymerase from transposon X-element	Catalytic. Deoxynucleoside triphosphate + DNA(n) = diphosphate + DNA(n+1).

associated with carapace width and the length of the 6th pleonite, the TASSEL analysis suggests the possibility of a polygenic effect on shape. This would not be a surprising result, as a number of recent studies have shown polygenic architecture for complex phenotypic characteristics such as morphometry (Laporte et al. 2015; Pallares et al. 2014; Hecht et al. 2012). Both TASSEL and LFMM analyses identified a number of markers associated with principal components of morphometric differences with most markers from TASSEL associated with the PC axes most heavily loaded with carapace width and abdomen length and width, a result that supports the TASSEL analysis on raw characters discussed above. In LFMM, the most markers were significantly associated with the two PC axes most heavily carapace, tail fan and leg characters. Both analyses identified markers associated with carapace and leg loaded principal component axes, suggesting that those measurements might be examined more closely in future efforts to discriminate between lobster stocks. Similarly with both analyses, few markers were significantly associated with claw-based characters, which have been utilized in the past to discriminate between lobster stocks (Cadrin 1995; Saila & Flowers 1969).

Although this is the first morphometric study linked to genomics in *H. americanus*, similar studies have been completed in the Pacific lamprey (Hess et al. 2014; Hess et al. 2012), European eel (Pujolar et al. 2014) and Lake whitefish (Laporte et al. 2015). These studies utilized similar genome wide association techniques to test for associations between SNP markers and morphological, environmental, and behavioral variables. One challenge, as identified in each study is the inherent complexity of

behavioral, physiological, and morphological traits and the likely interaction of many genes in the development and maintenance of such traits.

Sex-linked markers

The sex-linked markers were identified by both analyses as being heavily associated with sexually dimorphic characters in the claws and abdomen. Unlike many invertebrates, a number of macruran decapod crustacean species appear to have a WZ/ZZ mechanism for sex-determination (Legrand et al. 1987). In several of these species, the males are homogametic while the females are heterogametic (Parnes et al. 2003; Staelens et al. 2008). Although these findings are potentially substantial as they represent the first identification of a sex chromosome in *H. americanus*, they are beyond the scope of this manuscript and will be addressed at length in a later manuscript.

Function of genes associated with genetic markers

Though many markers showed significant associations with morphometry, it is difficult to assess their precise positions and exact function within *H. americanus* due to the lack of a reference genome. However, the BLAST analysis identified a number of markers associated with genes of various function in other species. Within the lobster, these genes likely have complex functions that are not yet known; however, a number have been studied and their functions identified in other organisms (Table 14). Of specific interest is the one locus identified as being under divergent selection in the ARLEQUIN and Bayescan analyses and correlating significantly to morphometric

differences as found by the LFMM analysis. This locus was revealed through a BLAST analysis to reside in or near a gene for the 40S ribosomal protein S3-A, a protein associated with cell growth, differentiation, and death in humans (Naora et al. 1998). There is, as of yet, no evidence that this gene has a corresponding role in lobsters; however, this finding deserves consideration in future studies. Should the gene have a similar effect in lobsters, and should variance at this gene impact morphometric differences between individuals and populations, then it is possible that divergent selection on the gene could result in morphometric differences.

As in most genome association research, especially in organisms without a reference genome, the associations found within these studies cannot be interpreted as the direct or indirect cause of morphological variation. Additionally, the markers identified through the two genome scan approaches cannot be automatically assumed to be directly involved but, instead, might be located near a gene that is under selective pressure. While the results preclude definitive conclusions as to the presence or absence of local adaptation in the American lobster, they do create the opportunity for future experimentation and bring up the issue of management of the observed biocomplexity.

The existence of morphological differences between groups of organisms has been utilized in the past to inform management decisions. In Atlantic cod (*Gadus morhua*), a species with limited genetic population structure (Knutsen et al. 2003; Jorde et al. 2007), management strategies are based on protecting diversity in fitness-oriented traits that have the potential to impact stock productivity (Olsen et al. 2008). These management strategies that incorporate a Darwinian approach, focus on phenotypic

differences between groups of individuals. Prior research has shown the need to manage biocomplexity in exploited species in order to manage genetic diversity and adaptive phenotypic plasticity (Olsen et al. 2008; Hutchings et al. 2007; Hilborn et al. 2003; Ruzzante et al. 2006).

This study highlights the existence of phenotypic variation in lobsters and, as such, the need for greater maintenance of small-scale diversity in the species. These management strategies may include a series of interconnected marine reserves as argued by Berkley et al (2004), which a modeling study by Baskett et al (2005) indicated could reduce some of the negative evolutionary impacts of fishery exploitation. Identifying these fisheries-induced phenotypic shifts requires consistent population monitoring including genetic and phenotypic assessments of the organisms and routine assessment of local ecosystems. Fisheries-induced evolution is especially important in species that take long times to mature and with significantly higher levels of fishery-based mortality than of natural mortality; including Atlantic cod and the American lobster (Jennings et al. 1999; Ault et al. 2005; Ernande et al. 2004). In the Atlantic cod, a period of rapid evolution of maturation rate immediately preceded the collapse of the fishery (Olsen et al. 2004). It is of critical importance to identify and regularly assess the phenotypic characters and adaptive genetic markers in the American lobster in order to be able to predict and potentially prevent a future fishery collapse as seen in the Atlantic cod.

Summary of findings

While the results of this study are certainly suggestive of at least some level of local adaptation, this study did not have sufficient coverage to rule out the possibility of hitchhiking with genes under selection nor did we identify the function of a majority of the genes associated with the SNPs identified. Without a reference genome and further experimentation, it is impossible to determine whether the 40S ribosomal protein S3-A in particular impacts, whether directly or indirectly, the morphometry of an individual.

Constant challenges to any of these experiments are the long life-span and slow growth of individual lobsters, the size of the lobster population and its habitat, and the size of its genome. Future studies on lobster population structure should encompass a range of ages (and sizes) and sample the same sites over several years.

Conclusion

As a culmination of six years of research and experimentation, the studies presented in this dissertation have furthered the field of research in the American lobster and have set the stage for future research in the fields of population genomics, local adaptation, and phenotypic plasticity. The behavioral recognition and morphometric differences found in *H. americanus* provide two potential mechanisms for selection of locally adaptive phenotypes due to assortative mating or selection on physical shape or underlying physiology. The development of a novel method of capturing and measuring lobsters with speed and with the ability to resample as needed will lead to the opportunity for larger sample sizes and, potentially, greater resolution of morphometry through use of more advanced techniques such as thin-plate spline (TPS) analyses (Park & Bell 2010).

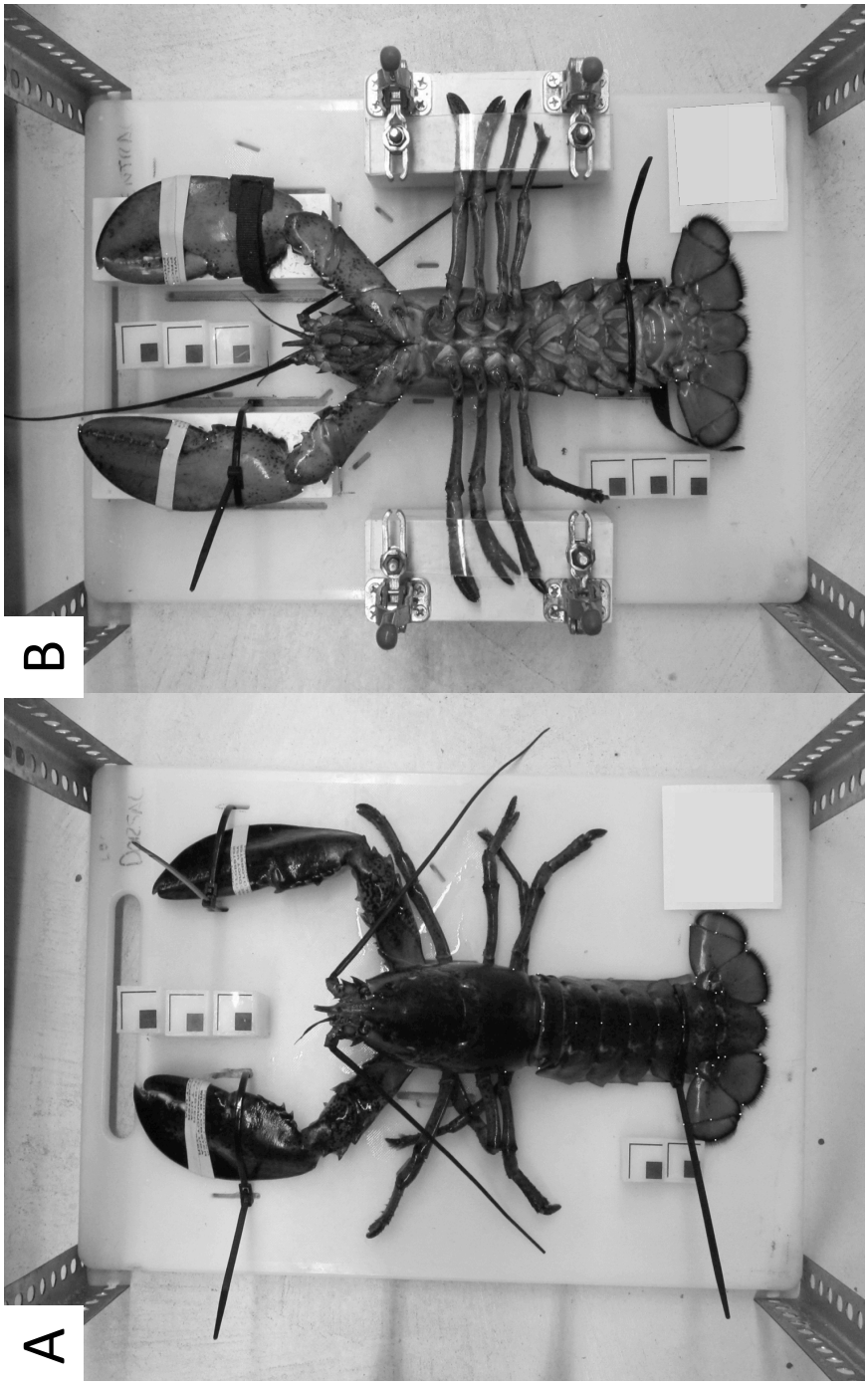
Population genetic analyses suggest that there are high levels of gene flow between lobster populations with little to no population structure seen in putatively neutral markers. To test hypotheses of plasticicy and local adaptation and to maximize the number of markers, candidate loci were identified through two genome scan and two SNP association techniques. Comparison of these loci to the lobster transcriptome and the NCBI database suggest a functional role of the loci, although without a reference genome of the lobster or a closely related species, those roles are largely unknown.

Future studies in this field should build upon the results of this study as this research has set the groundwork for a number of next-step studies. Specifically, I would suggest the following experiments to further the field of research. 1) A multi-year analysis of genetic and morphometric population structure in *H. americanus* using lobsters of varying sizes including larval and juvenile lobsters. The sample sizes of this analysis must be substantially larger than within the present study to provide enough power due to small F_{ST} values. 2) A series of common-garden experiments to assess the ability of lobsters to respond plastically to environmental differences. Due to the challenges of controlling for environmental variables and the risk of genetic contamination that would exist in situ, this experiment would likely be most effective using young lobsters with shorter inter-molt periods in a laboratory setting. Environmental variables should include diet, temperature, relative light levels, and benthic habitat. Of course, none of these studies will be able to truly make concrete conclusions without the existence of an annotated reference genome in *H. americanus* or a closely related species. For this reason, it is critical that a reference genome is

constructed in the near future. The lobster fishery in New England adds significantly to both the local economy and its associated culture. To preserve the fishery, it is especially important to truly understand the underlying genetic population structure and identify the biotic or abiotic mechanisms that create or inhibit the separation of groups of individuals.

APPENDIX 1

Images taken of dorsal (A) and ventral (B) surfaces of the lobster to be measured. Calibration blocks were used to account for variance in height of different morphometric characters.



APPENDIX 2

Markers identified as putatively under divergent selection in ARLEQUIN and Bayescan and identified as associated significantly with PC axes in LFMM and TASSEL. *Note: The PC axes in LFMM and TASSEL are not identical. See text for details.*

Marker	Arlequin	Bayescan	LFMM	TASSEL GLM	TASSEL MLM
1-22921	YES				PC2
1-24252				PC2	PC2
1-55169	YES	YES			
1-122545	YES	YES			
1-169861					PC2
1-171491				PC2	PC2
1-197669			PC3		
1-197958	YES		PC1		
1-209103			PC3		
1-287004				PC2	PC2
1-355188					PC2
1-366334	YES				
1-382228				PC2	
1-424624					PC2
1-434792				PC4	
1-442857			PC1		
1-463671				PC2	PC2
1-474876				PC2	
1-479711			PC1		
1-505260				PC2	PC2
1-508604			PC1		
1-510707					PC2
1-526286				PC2	PC2
1-535978	YES				
1-567707		YES			
1-581890				PC2	PC2
1-599026				PC2	
1-633351				PC2	
1-639579	YES				
1-665346	YES				
1-703660				PC4	PC4
1-719333	YES		PC3		

Marker	Arlequin	Bayescan	LFMM	TASSEL GLM	TASSEL MLM
1-727552			PC1	PC2	PC2
1-746043			PC1		
1-746056			PC1		
1-755792				PC2	PC2
1-763655				PC2	PC2
1-773932	YES				
1-784937				PC2	PC2
1-785243	YES				
1-795880	YES				
1-808937	YES				
1-863689	YES				
1-938496	YES	YES			
1-992906	YES	YES			
1-998993			PC1		
1-999040			PC1		
1-1001832				PC2	PC2
1-1026609	YES				
1-1026713				PC2	PC2
1-1059521			PC1		
1-1067823	YES				
1-1090744				PC2	PC2
1-1108695				PC2	PC2
1-1195716			PC1, PC2		
1-1211269	YES	YES			
1-1213083	YES				
1-1232641	YES				
1-1235018				PC2	PC2
1-1263039	YES				
1-1263275			PC1		
1-1264384	YES				
1-1280938			PC1		
1-1306058			PC1		
1-1387200				PC2	PC2
1-1392899	YES				
1-1401939			PC1		
1-1401949			PC1		
1-1409295				PC2	PC2
1-1409826				PC2	PC2

Marker	Arlequin	Bayescan	LFMM	TASSEL GLM	TASSEL MLM
1-1443620	YES	YES			
1-1478449	YES				
1-1528275	YES				
1-1544937		YES			
1-1630665	YES				
1-1630711	YES				
1-1649793			PC1		
1-1680144				PC2	PC2
1-1713801				PC3, PC4	PC4
1-1753112			PC3		
1-1753112			PC1		
1-1757708				PC4	
1-1758143				PC2	PC2
1-1770875				PC2	PC2
1-1794022	YES				
1-1831504			PC1		
1-1881200	YES				
1-1909130				PC2	PC2
1-1951841				PC3, PC4	PC4
1-1963022				PC2	PC2
1-1976852			PC1		
1-1989187			PC3		
1-2009735				PC2	PC2
1-2033018				PC3, PC4	PC4
1-2039621	YES	YES	PC3		
1-2061519	YES				
1-2061854				PC2	PC2
1-2082866			PC1		
1-2083439					PC2
1-2093876	YES				
1-2142240	YES				
1-2151856				PC2	PC2
1-2152022					PC2
1-2159536	YES				
1-2171272			PC1		
1-2223634				PC2	
1-2241515	YES	YES			
1-2256812	YES	YES			

Marker	Arlequin	Bayescan	LFMM	TASSEL GLM	TASSEL MLM
1-2263643	YES	YES			
1-2291498	YES				
1-2319089				PC2	PC2
1-2341745				PC3, PC4	PC4
1-2387905		YES			
1-2416008			PC1		
1-2504766				PC2	PC2
1-2516260				PC2	PC2
1-2535196	YES	YES			
1-2539103				PC2	PC2
1-2599180	YES	YES			PC2
1-2669880				PC2	PC2
1-2690767	YES				
1-2705412	YES				
1-2723870			PC3		
1-2734847			PC1		
1-2761342				PC2, PC4	PC2, PC4
1-2772828	YES	YES			
1-2824530			PC1		
1-2830951			PC1		
1-2834557	YES	YES			
1-2837665			PC1		
1-2841034				PC2	PC2
1-2847439			PC1		
1-2848872	YES				
1-2853963	YES	YES			
1-2879520				PC4	PC4
1-2892681	YES				
1-2895566			PC3		
1-2947688			PC1		
1-2952152	YES				
1-2958180	YES				
1-3009939			PC1		
1-3010338	YES		PC3		
1-3011726	YES	YES	PC2		
1-3013625			PC1		
1-3042131	YES				
1-3042637				PC2	PC2

Marker	Arlequin	Bayescan	LFMM	TASSEL GLM	TASSEL MLM
1-3208943					PC2
1-3235689	YES				
1-3282361	YES	YES			
1-3321946	YES				
1-3325910	YES				
1-3457556			PC3		
1-3500523			PC1		
1-3533546	YES				
1-3534313				PC3, PC4	PC4
1-3601139	YES				
1-3616025			PC2		
1-3650351	YES				
1-3734873	YES				
1-3741157	YES				
1-3755092					PC2
1-3762770	YES	YES			
1-3815156				PC2	PC2
1-3818041	YES				
1-3847799	YES				
1-4514294			PC2		
1-4514304	YES				

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